

A continuum-based reaction-diffusion model for spread of gene silencing in chromosomal inactivation

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ABSTRACT

Regulation of gene silencing in large chromosomal regions is crucial for development and disease progression. One example of massive gene silencing is X chromosome inactivation (XCI), a process essential for gene dosage compensation. During XCI, most genes in the chromosome are inactivated following the transcription of long noncoding RNA XIST. Recent experiments showed that the spread of silencing is restricted in space but the mechanism of controlling the spread remains unclear. Here, we develop a continuum-based, reaction-diffusion model that elucidates chromosomal inactivation through a regulatory network for XIST-mediated gene silencing. We find that the spread of XIST can be tuned by known negative feedback loops regulating its synthesis and degradation, and that the spread of gene silencing is controlled by a wave-pinning mechanism driven by global regulation of silencing complex together with local epigenetic regulators. We use a 3D chromosome structure inferred from experimental data and our modeling framework to show the spatiotemporal regulation for spread of gene silencing. Our method enables the investigation of the inactivation dynamics of large regions of chromosomes with varying degrees of the spread of gene silencing. Our model provides mechanistic insights that quantitatively relate gene regulatory networks to the tunability and stability of chromosomal inactivation.

Keywords: X chromosome inactivation; XIST; gene silencing; long noncoding RNA; reaction-diffusion model

INTRODUCTION

Gene silencing in large chromosomal regions is essential for the development of mammals. Particularly, X-chromosome inactivation (XCI) is used to achieve dosage compensation in female cells to balance the expression of X-linked genes of the two sexes (Payer and Lee 2008). Before mammalian XCI, one of the two X chromosomes in each cell of the early female embryo is randomly chosen for inactivation. During XCI, the expressions of X-linked genes in the X chromosome selected to be inactivated (Xi) are repressed in a progressive manner upon the transcription of XIST, an X-linked, long-noncoding RNA (lncRNA) (Kay et al. 1993; Heard et al. 1997). XIST molecules are tethered to multiple chromosomal locations, where they recruit polycomb repressive complexes (PRCs) for gene silencing (Sunwoo et al. 2015). The spread of gene silencing was shown to be influenced by the locations of the genes in both one-dimensional (1D) genomic coordinates and

three-dimensional (3D) genome structures. The completion of XCI results in the stable repression of most, but not all, genes in the X chromosome (Balaton and Brown 2016). The number of X-linked genes that remain to be active after the completion of XCI (i.e., the XCI escape gene, or escapees) can vary across tissues and species (Berlitch et al. 2015; Tukiainen et al. 2017). The mechanism controlling the extent of gene silencing spread, which in turn determines the escape genes, in XCI remains elusive.

Several key features of XIST-mediated gene silencing were recapitulated with integrations of XIST transgenes into autosomes (Lee and Jaenisch 1997; Minks et al. 2013; Naciri et al. 2021). These studies showed the ability of the induced XIST expression in an autosome to silence genes in the adjacency of the XIST integration/transcription site in a continuous fashion over genomic locations. Furthermore, the spread of gene silencing is restricted in space at steady state (Minks et al. 2013). Similar to the question of determinants of escape genes, factors

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contributing to the gene silencing spread upon transgene XIST induction are unclear.

Previous mathematical models based on ordinary differential equations (ODEs) describing gene regulatory networks have provided useful insights into the mechanisms of the decision-making process of XCI for robustly selecting one X chromosome in each cell (Li et al. 2016; Mutzel et al. 2019). In addition, agent-based modeling has been used to show the dynamic distribution of XIST molecules over the 3D structure of the X chromosome (Lappala et al. 2021). However, these modeling frameworks did not capture the connection between the dynamics of XIST distribution and that of gene silencing. The latter is controlled by complex regulatory networks containing feedback loops in a spatially dependent manner. These feedback loops include the transcriptional and post-transcriptional self-regulations of XIST (Rodermund et al. 2021; Jachowicz et al. 2022) as well as epigenetic modifications that confer gene activity switches and memory which have been described in previous models (Dodd et al. 2007; Zhang et al. 2014; Ramakrishnan et al. 2022). The lack of quantitative description of these interconnected regulatory elements has been limiting our understanding of XIST-mediated gene silencing in both natural and engineered systems.

Various pattern formation mechanisms have been successfully used to explain crucial regional boundaries at the cellular and the subcellular levels, including cell polarity establishment. In particular, a combination of a positive feedback loop and the chromosome-wide limitation of key diffusive, feedback-driving molecules can produce a “pinned” wave that represents a stable boundary between an active and an inactive subcellular region (Mori et al. 2008). Because of the presence of numerous feedback loops and diffusive regulators for chromatin activities, we hypothesize that such a pattern formation mechanism may contribute to the range and stability of X chromosome inactivation.

In this work, we used a continuum-based reaction-diffusion framework to model XIST dynamics and gene silencing induced by XIST upregulation during XCI. We show that the feedback loops of XIST’s transcription and degradation have significant impacts on the steady state distribution of XIST and the speed to attain the distribution. We describe a wave-pinning mechanism based on chromatin-level feedback between histone modifications and repressive complexes, and the proposed mechanism offers a plausible explanation for controllable spread of gene silencing in the X chromosome. In addition, this generic version of our model gives insights into lncRNA-mediated gene silencing in autosomes. Interestingly, both chromosome-wide regulation of silencing complex and local variations of histone modifications can contribute to wave-pinning. We extended the continuum model to capture 3D chromosome structure and showed the utility

of our model in explaining key observations such as escape genes under realistic spatial constraints. Overall, our continuum-based reaction-diffusion model integrates regulatory components contributing to XCI in a systems approach, and it provides new mechanistic insights into factors controlling the spread of lncRNA-mediated gene silencing.

RESULTS

The roles of feedback loops controlling XIST expression on dynamics of gene silencing

To understand how regulations of XIST can influence the XIST distribution, we first built a spatiotemporal model with a one-dimensional (1D) domain and a reaction-diffusion system based on partial differential equations (PDEs). The domain can be viewed as a chromosome region projected onto a 1D space (we will consider a more realistic spatial setting later in this manuscript). A key difference between this model and previous models is that our model allows the diffusion of molecules in continuous space and time, which in turn permits flexibility of molecular movements and descriptions of complex gene regulatory networks. The latter advantage allows us to use a simple set of differential equations to study the effects of molecular concentrations and interactions on gene silencing. Specifically, we first considered a spatiotemporal module of regulations for XIST transcription, degradation and diffusion. We used a dimensionless PDE,

$$\frac{\partial [\text{XIST}]}{\partial t} = \frac{\beta_X}{1 + \left[\frac{[\text{XIST}]}{\phi_\beta}\right]^{n_\beta}} - \frac{\alpha_X \left[\frac{[\text{XIST}]}{\phi_\alpha}\right]^{n_\alpha}}{1 + \left[\frac{[\text{XIST}]}{\phi_\alpha}\right]^{n_\alpha}} [\text{XIST}] + D_X \nabla^2 [\text{XIST}], \quad (1)$$

to describe these regulations. Here, $[\text{XIST}]$ represents the concentration of XIST molecules, β_X is the basal transcription rate of XIST, α_X is the basal degradation rate constant, D_X is the diffusion coefficient, ϕ_β is the threshold of the inhibition of XIST’s transcription by itself, n_β describes the nonlinearity of this transcription feedback (Jachowicz et al. 2022), ϕ_α is the threshold of the activation of XIST’s degradation by itself, and n_α describes the nonlinearity of this degradation feedback (Rodermund et al. 2021). Both feedback loops are experimentally observed negative feedback loops (NFLs) in which XIST can limit the level of itself (Fig. 1A). We selected biologically plausible parameter values to examine the representative dynamical behaviors of the gene regulatory networks (see [Supplemental Material](#)). Importantly, the roles of these feedback loops on distributions of XIST and the subsequent gene silencing were unclear due to the lack of

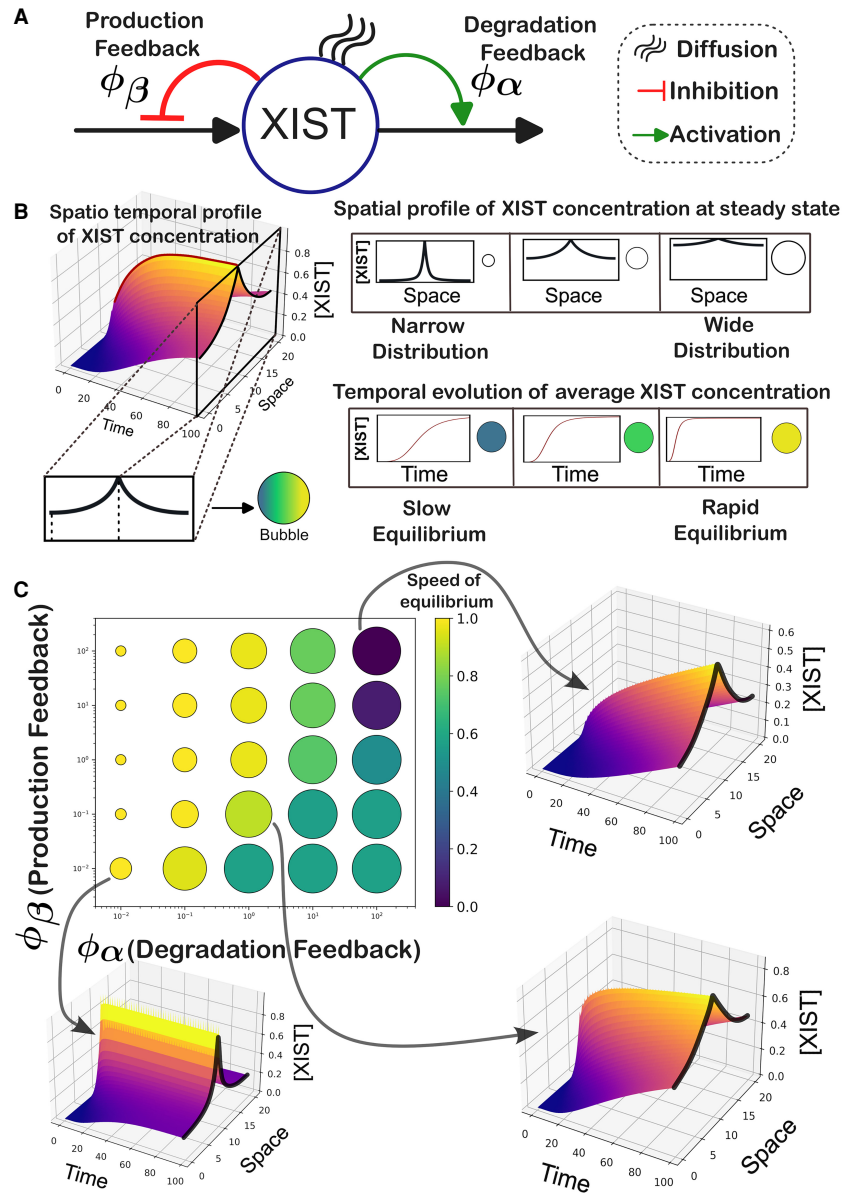


FIGURE 1. XIST module and its simulation results. (A) A regulatory network for controlling XIST levels. This network was used to construct a PDE model to examine the time-dependent spatial distribution of XIST. Two feedback loops at degradation and transcription levels are controlled by two threshold parameters ϕ_α and ϕ_β , respectively. (B) Simulation of the 1D PDE model (example result shown on left), and two metrics assessing the width and the speed of XIST spread (right). (C) A grid search for the strengths of the feedback loops (small ϕ corresponds to lower threshold and stronger feedback) and the associated results of XIST spread width and speed. In these simulations, $n_\beta = n_\alpha = 4$, $\alpha_X = \beta_X = 1$, and $D_X = 1$.

rigorous models. To examine NFLs' function in a quantitative framework, we scanned values of ϕ_β and ϕ_α in a grid space with a simple assumption of all other parameters (Fig. 1B,C). We asked whether the two NFLs can determine how far XIST can reach in the 1D domain with substantial concentration with respect to its level at the transcription site, and how fast XIST distribution can obtain a steady state (Fig. 1B). Overall, the range of the steady state distribution is negatively correlated with the speed of equilibrium attainment (Fig. 1C, smaller circles tend to be brighter). However, we found that moderate levels of both feed-

backs gave rise to broad distributions of XIST and relatively rapid attainment of equilibrium (e.g., Fig. 1C, lower right plot; Supplemental Figs. S1–S3). It should be noted that the broad distribution and rapid equilibrium performance can be achieved simply by increasing the diffusion coefficient of the molecule. However, this way of obtaining the performance would require substantial changes of the physical properties of the molecule (e.g., size). Therefore, our results suggest a powerful approach of tuning the dynamical distribution of XIST by expression regulations alone.

Tunability of the spread of gene silencing by *cis*- and *trans*-acting factors

Next, we focus on a module downstream from XIST that is responsible for the spread of gene silencing. Recent experiments suggest that polycomb repressive complexes (PRCs) 1 and 2, both critical for XCI (Dixon-McDougall and Brown 2021; Masui et al. 2023), are involved in reciprocal regulations with histone posttranslational modifications. Specifically, PRC2 is activated by its enzymatic product H3K27me3 (Lee et al. 2018). Likewise, histone H2A monoubiquitination (H2Aub) binds and stimulates PRC2 (Kalb et al. 2014), while H2Aub is an enzymatic product of PRC1 which in turn can be recruited by PRC2 (Fischle et al. 2003; Min et al. 2003). These experiments support a positive feedback loop between repressive complexes and the gene-silencing permissive posttranslational modifications of histones. We use the following PDEs to describe the dynamics of Polycomb repressive complex PRC2 (as a representative factor for chromatin modification and a transcription repressor) in response to XIST activation:

$$\begin{aligned}\frac{\partial u_1}{\partial t} &= f(u_1, u_2) + \beta_{u_1} - \alpha_{u_1} u_1 + f_s([XIST]) + D_{u_1} \nabla^2 u_1 \\ \frac{\partial u_2}{\partial t} &= -f(u_1, u_2) + \beta_{u_2} - \alpha_{u_2} u_2 - f_s([XIST]) + D_{u_2} \nabla^2 u_2 \quad (2) \\ \frac{\partial g}{\partial t} &= \frac{\beta_g}{K^2 + u_1^n} - \alpha_g g,\end{aligned}$$

where $f(u_1, u_2) = u_2[\kappa_0 + \gamma u_1^n / (K^2 + u_1^n)] - \delta u_1$, u_1 is concentration of the active form of PRC2, u_2 is the concentration of the inactive form of PRC2. g represents the activity of any gene in a region of chromosome. $f(u_1, u_2)$ represents the interconversion rates between the two forms of PRC2 (Fig. 2A). The conversion from the inactive form to the active form is assumed to be influenced by the active PRC2 via a positive feedback loop, which is supported by the conformational change of the complex induced by H3K27me (Lee et al. 2018; Stafford et al. 2018), a histone modification catalyzed by PRC2. The feedback is characterized by γ , the maximum conversion rate controlled by the feedback, K the threshold of self-activation, and n the Hill exponent controlling the nonlinearity of the feedback. κ_0 is the basal conversion rate constant from u_2 to u_1 , and δ is the conversion rate constant from u_1 to u_2 . α_{u_1} and α_{u_2} are the outflux (e.g., disassembly) rates of the active and inactive forms of PRC2, respectively. β_{u_1} and β_{u_2} are the influx rates (e.g., assembly rate) of the active and inactive forms of PRC2, respectively. D_{u_1} and D_{u_2} are the diffusion coefficients of the active and inactive forms of PRC2, respectively. g is the activity of a representative target gene controlled by PRC2. The function $\beta_g / (1 + u_1)$ describes the rate of gene deactivation triggered by u_1 , where β_g is the basal activity. α_g is the relaxation rate constant of the gene activity. In the scope of this study, the status of chromatin inactivation is described by the active form of PRC2 (u_1) because g is simply controlled by PRC2

negatively, but we included the variable g to emphasize the goal to study gene inactivation in our work. f_s is the function describing how XIST transiently influences the PRC2 activation (see details below and the rationale for choosing parameter values in the [Supplemental Material](#)).

To better understand our model in a stepwise fashion, we modularized our model by separately considering the dynamics of PRC2 complex and XIST. We first focus on a *chromatin module* describing PRC2 and gene activities (Eq. 2) which does not include XIST explicitly (Fig. 2A), and f_s in Equation 2 is assumed to be a transient, imposed stimulation at the beginning of the simulation (e.g., $1 < t < 4$) that only occurs in the middle of the spatial domain (see [Supplemental Material](#) for details). The dynamics of XIST will be considered more carefully in a later section when we integrate two modules (Eqs. 1 and 2). With the chromatin module, we simulated the model in a 1D chromosome domain (Fig. 1B), and we introduced a local perturbation in the middle of the domain (presumably triggered by tethering of XIST to chromatin) where the active PRC2 complex level was elevated while the rest of the region has a low concentration of active PRC2 complex (i.e., a high level of gene expression). As we expected, the positive feedback loop resulted in a trigger wave (also called a traveling wave) of gene silencing (Fig. 2B), which stems from local bistability of the chromatin states ([Supplemental Fig. S4](#)). This trigger wave gradually expands the active PRC2-high state to the entire region. Trigger waves driven by positive feedback were widely studied in other biological systems (Gelens et al. 2014), and our model suggests a new utility of this mechanism in chromosomal inactivation. Nevertheless, we found that the gene silenced state covered the entire region at steady state even when we simulated a larger domain ([Supplemental Fig. S5](#)). While this shows the robustness of the trigger wave, the result is inconsistent with the observation that chromosomal inactivation only occurs in one part of the X chromosome during development (Tukiainen et al. 2017), and in an even more restricted region when transgene XIST is activated in an autosome (Minks et al. 2013; Naciri et al. 2021). We therefore asked what can cause the restriction of the spread of gene silencing. Interestingly, reducing the strength of the positive feedback loop in flanking regions of the chromosome resulted in an abrupt termination of the trigger wave (Fig. 2C). This is consistent with previous reports on sequence and epigenetic determinants for XIST-mediated gene silencing (Tang et al. 2010; Loda et al. 2017). Nonetheless, these local features cannot fully explain the variable extents of the gene silencing spread during XCI and activation of transgene XIST (Tukiainen et al. 2017). We therefore tested a global mechanism, defined as chromosome-wide mechanism here, for wave pinning. By moderately lowering the influx (e.g., production/assembly) and the outflux (e.g., degradation/disassembly) rate constants of PRC2 (α and β in Eq. 2), we found that the spread of

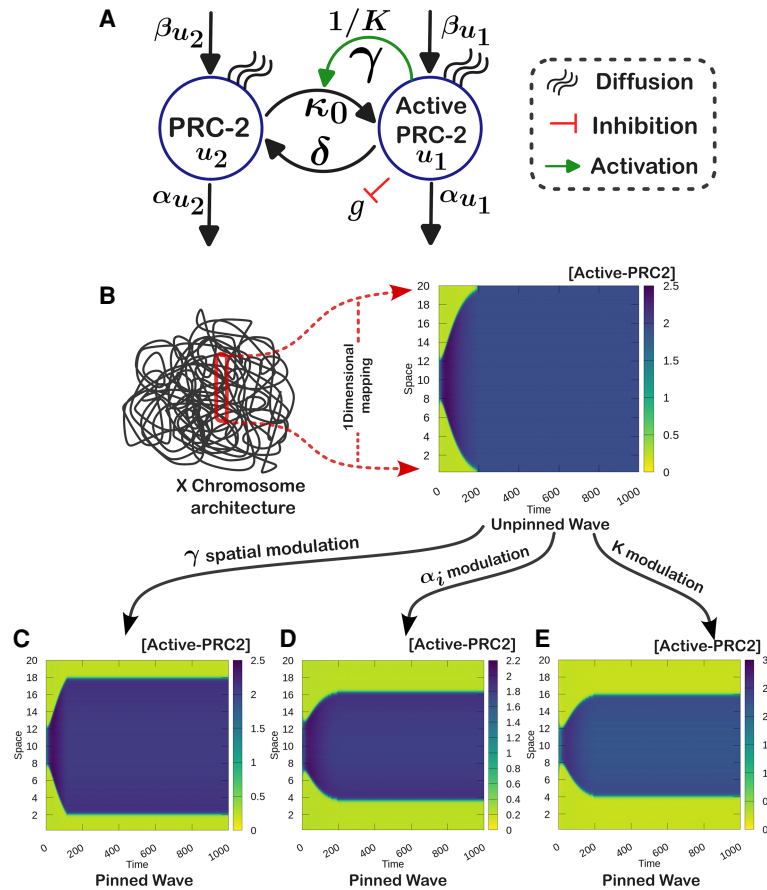


FIGURE 2. Chromatin module and its simulation results. (A) A regulatory network for controlling inactive and active PRC2 levels. This network was used to construct a PDE model to examine the time-dependent spatial distribution of inactive and active PRC2 molecules. (B) Schematic illustration of the mapping procedure of certain chromosomal regions in one spatial dimension. The heat map represents the time evolution of the active PRC2 wave leading to homogeneous spatial profile (where the blue color represents the inactive regions, and the green color represents the active regions of the chromosome). (C) Attainment of spatial heterogeneity in terms of pinned wave by spatially modulating the more sensitive feedback coefficient γ . (D) Visualization of the pinned wave achieved through enhancement of degradation coefficient α_x relative to the unpinned state. (E) A pinned wave realized by increasing the value of the feedback threshold parameter K , which has a lower sensitivity toward the dynamics of XIST molecules. Parameter values for the simulations in this figure are listed in Supplemental Table S1.

gene silencing can be restricted by a limited amount of PRC2, which gave rise to a fraction of silenced chromosomal region at the steady state (Fig. 2D). While this mechanism of globally limited regulatory components was related to the one used for explaining the robust polarized distribution of molecules at the cell membrane, where the concentration of a regulator was assumed to be a constant (Mori et al. 2008), our model allows both dynamic changes and flexible initial distributions of PRC2 concentrations, which is applicable to broader biological contexts. A similar assumption was recently used to show that limiting the total cellular concentrations of histone modification enzymes is necessary for maintaining epigenetic memory during cell divisions (Owen et al. 2023). Note that our model allows

general influx and outflux of PRC2, which can be influenced by exchange of subunits between chromosomes. Therefore, PRC2 can be distributed across multiple chromosomes at steady state in our framework. We found that the strength of the positive feedback loop is important for the wave pinning (Fig. 2E), and for the position where the wave is pinned (Supplemental Fig. S6). We expect that biological systems such as XCI utilize both local (Fig. 2C) and global (Fig. 2D) mechanisms to shape the boundaries of repressed/active chromosomal regions. We tested a combination of these two mechanisms and showed that both abrupt and gradual spreads of chromosomal silencing can be produced with this more realistic combination (Supplemental Fig. S18). The relative weights of local and global factors contributing to wave-pinning can be context-specific. For example, rapid influx of PRC2 from other nuclear regions or elevated assembly rates due to feedback from nucleus-wide chromatin remodeling can lower the restriction from the global mechanism, thereby allowing more prominent regulations by local epigenetic factors. In terms of kinetic phenomena that distinguish the two mechanisms, a linear spatio-temporal spread of chromatin silencing and an abrupt wave pinning reflect a locally dominated mechanism, whereas a nonlinear spread of a silencing wave and a gradual wave-pinning are more likely caused by a global mechanism (Fig. 2C,D).

This distinction can be used in future experiments to examine the mechanisms.

In summary, our results provide a new mechanistic explanation, that is, a wave-pinning model, for the spread of gene silencing at the chromosomal level, and we identified two distinct factors contributing to the spatial restrictions of the wave. We expect that biological processes such as XCI use a combination of local and global factors for the precise control of gene silencing waves.

Spread of gene silencing from multiple initiation sites

Previous studies have shown that XIST binds to multiple sites on the X chromosome, and these sites are the places

where the spread of gene silencing begins (Simon et al. 2013). To study potential interactions of multiple silencing waves, we tested the wave dynamics initiated from two sites presumably bound by XIST at the beginning of XCI. Note that here we are still not considering the dynamics of XIST explicitly in this chromatin module. Instead, we introduced two transient stimulations in the simulated spatial domain described in Equation 2. We focused on a wave pinning mechanism based on global control of polycomb repressive complexes because the characteristics of wave pinning driven by local parametric changes are predictable intuitively. Unsurprisingly, with two local stimulations for gene silencing, we observed two silencing waves, each starting to spread in both directions in the chromosome region at the beginning of the simulation. However, the two waves became asymmetrical and repelled the propagation of each other (Fig. 3A, left heat map). To examine the mechanism underlying this phenomenon, we quantified the levels of both active and inactive PRC2 at an early stage of the simulation (Fig. 3A, heat maps and line plots). When the active PRC2 levels were still symmetrical with respect to the locations of the stimulations (Fig. 3A, orange dashed vertical lines), the inactive PRC2 levels started to become asymmetrical: the region between the two stimulations had a lower concentration of inactive PRC2 compared to the exterior regions, due to the depletion of inactive PRC2 induced by two waves, as opposed to one wave in each exterior region (Fig. 3A, green curves).

We next asked whether silencing waves can influence each other when they are located in two separate chromosomal regions. Unlike the models presented earlier in this work, the two regions correspond to two chromosomal segments that are not adjacent to each other in genomic coordinates but are relatively close in 3D space. To simulate this in a 1D framework, we created nonchromosomal space that allows diffusion of molecules but cannot be involved in any feedback-related reactions (Eq. 2) (white space in Fig. 3B). When we placed the two segments, each perturbed by a wave-enabling stimulation, with a long distance between them, we found that the two waves did not influence each other's dynamics (Fig. 3B). However, when the two segments were close, the two silencing waves repelled each other as we saw with the one-segment model (Fig. 3C). We next asked whether the movement of chromosomal regions can influence the silencing waves. We simulated a scenario of two chromosomal regions approaching each other, mimicking a chromosome undergoing compaction (Supplemental Fig. S7), and we found that the steady state silencing distribution was determined by the steady state, but not transient, positions of chromosomal regions (Fig. 3D). Furthermore, the repulsion of the two silencing waves was observed for both the no-flux boundary condition, which was used for all simulations presented so far, and the periodic boundary condition (Supplemental Figs. S8, S9). This shows that the

interactions of waves were not simply due to the choice of boundary conditions and that these interactions may occur in higher-dimensional space. Furthermore, multiple silencing waves were observed when diffusion coefficients varied between chromatin-dense regions and void space (Supplemental Figs. S10, S11; Supplemental Table S5). In summary, our results show that the control of gene silencing via global levels of repressive complexes can give rise to asymmetrical silencing wave propagation, and interactions of multiple waves under both static and dynamic conditions of chromosomal regions' positions.

Integration of the XIST and the chromatin modules

We next combined the XIST module (Eq. 1) and the chromatin module (Eq. 2) to build an integrated system for studying XCI-mediated gene silencing (Fig. 4A). To bridge the gap between the dynamics of XIST concentration and the concentration of the repressor complex, we used an intermediate variable to represent the status of the tethered XIST-protein complex (Pandya-Jones et al. 2020). A higher concentration of XIST triggers the activation of the tethering sites in a probabilistic manner (see Fig. 4B, left and middle panels; Supplemental Material). The locations of the tethering sites were randomly chosen in the simulated space (see the next section for optimization of the tethering sites). The activation of the tethering sites first leads to the activation of the PRC2 complex in the adjacency of the sites, which in turn initiates multiple trigger waves (Fig. 4B, right panel). At the steady state, most of the simulated chromosomal region was silenced, but due to the pinning of the waves, some regions remained to be active, reflecting the stable expression of the genes that have escaped from the chromosomal inactivation. In this model, we assumed spatially homogeneous feedback strengths to examine the effect of global regulations on the silencing wave. Interestingly, some asymmetrical wave patterns were produced due to the random distribution of the tethering sites and the resultant asymmetric interactions among waves that we explained in the previous section (Figs. 3, 4). As expected, using an imposed, rather than random set of tethering sites, possibly governed by local chromatin features, produced equivalent results (Supplemental Fig. S15; Supplemental Table S6). Overall, the combined model suggests that the XIST-mediated gene silencing can be captured by a dynamical system with multiple traveling waves, and such waves are initiated by both high local concentration of XIST and the existence of local protein assemblies at selected sites of the chromosome. Previous work showed that the nucleation of individual XIST molecules with interacting proteins can produce foci that ultimately influence the sites of silencing propagation (Markaki et al. 2021). We expect that such a phase separation mechanism is crucial for determining the locations of the repression-enabling tethering sites of the XIST for wave initiation

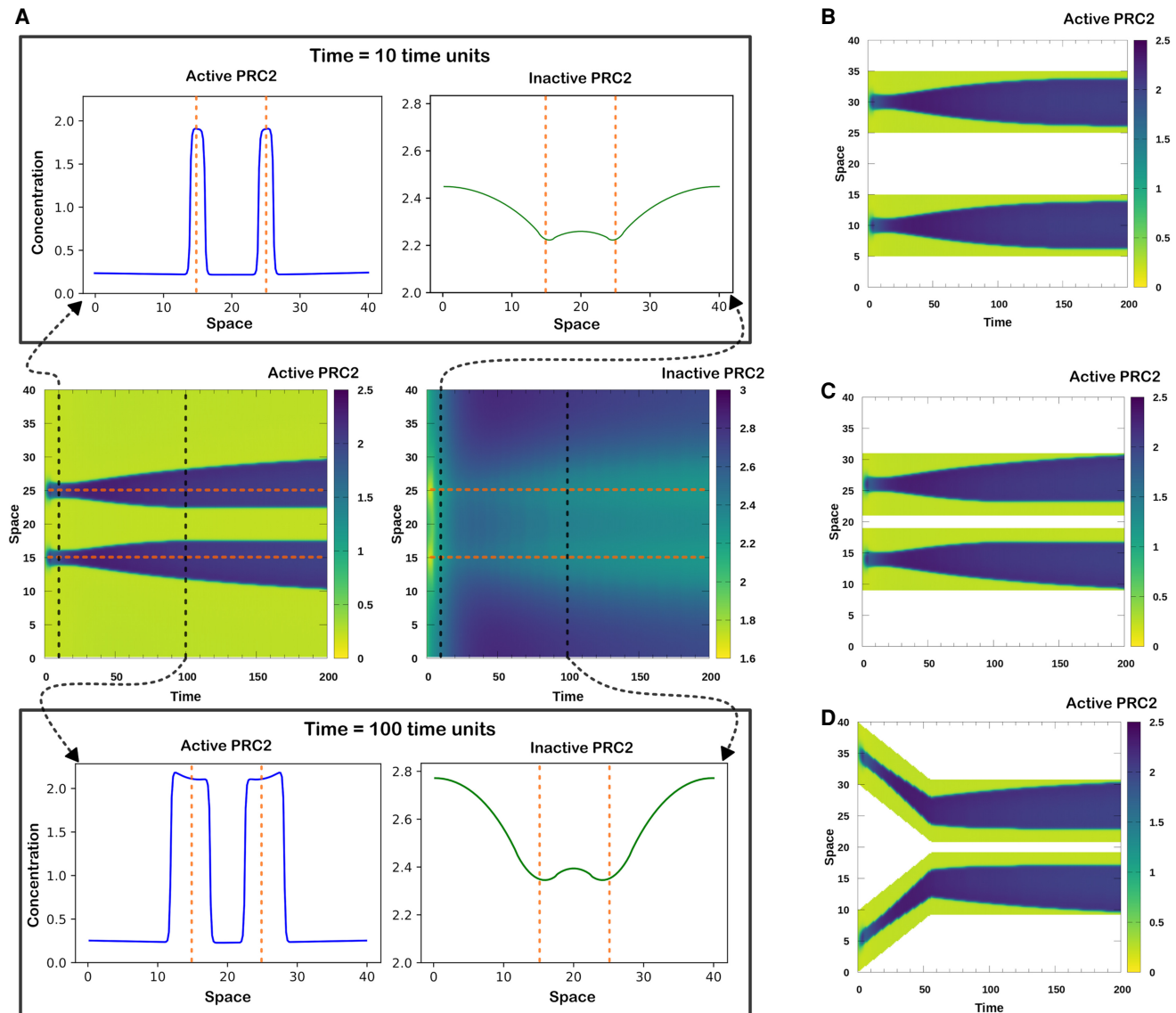


FIGURE 3. Interaction among the silencing waves and its analysis. (A) The heat maps represent spatiotemporal profile of both active and inactive PRC2 which clearly reveals the repulsive interaction between silencing waves and resulting symmetry breaking. A more detailed analysis of the spatial distribution of both molecules at two different time points unravels the unequal distribution of inactive PRC2 as the origin of symmetry breaking during the movement of silencing wave fronts. (B–D) Illustration of the effect of chromosomal movement on spatial profile of active PRC2 complex where the white, blue and green regions represent the void space, silenced and active chromosomal regions, respectively. The results indicate that the steady state distribution of the molecules is highly dependent on the final spatial orientation of the chromosome. Parameter values for the simulations in this figure are listed in Supplemental Table S2.

proposed in our model, whereas the wave pinning mechanism is a distinct approach to shape the stable boundaries of the inactivated regions of the chromosome.

A 3D model for spatiotemporal dynamics of chromosome inactivation

To illustrate the utility of our modeling framework with realistic chromosome structures and experimental data, we built a proof-of-concept 3D model for the X chromosome

and its inactivation. We first used a previously published 3D model of the X chromosome inferred from Hi-C sequencing data (Lappala et al. 2021). While the X chromosome structure is dynamic during XCI, we chose the inactive X chromosome structure because we showed with the 1D model that the stationary phase of the chromosome structure is the primary spatial factor determining the silencing waves (Fig. 3). In addition, since the inferred structure represents a representative configuration across multiple cells and multiple time points at the stationary

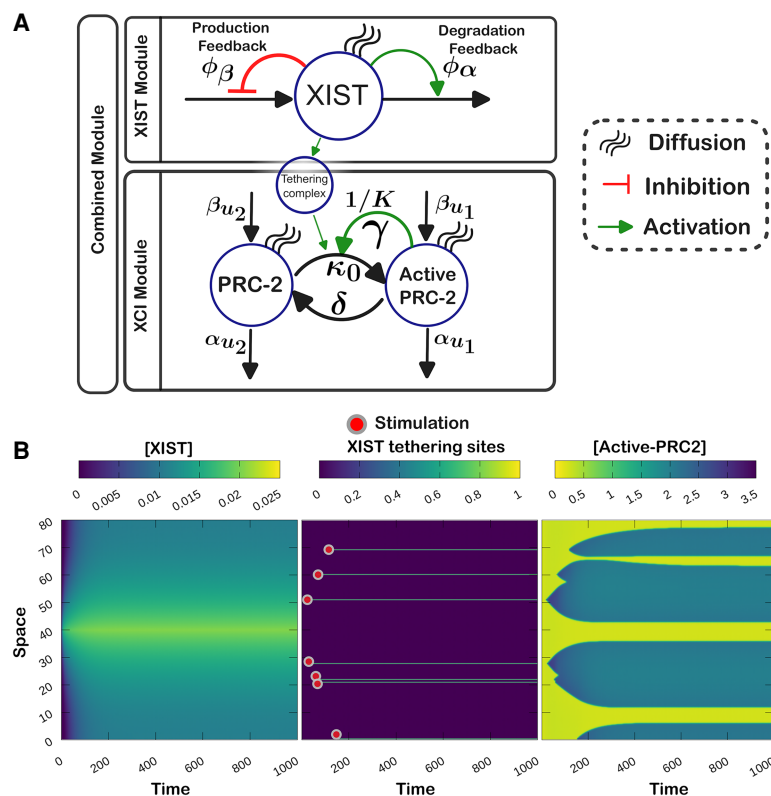


FIGURE 4. Integrated module and the simulation results. (A) Illustration of the combined biochemical network governing the underlying spatiotemporal dynamics of XIST, active PRC2 and inactive PRC2 molecules. The XIST module and X chromosome inactivation module are bridged by the tethering module that incorporates the effect of XIST spatiotemporal profile to the dynamical landscape of X chromosome inactivation. (B) The simulation results presented in this subpanel depict the spatiotemporal profile of XIST molecules (left), the tethering sites of the XIST molecules leading to stimulations (center), and the time evolution of the chromosomal inactivation profile (right). A detailed description of the parameters used for the simulation is provided in the Supplemental Material. Parameter values for the simulations in this figure are listed in Supplemental Table S3.

phase, we introduced a continuous spatial parameter γ that determines strength of the feedback between chromatin and the repressive complex, reflecting the duration or likelihood of chromatin occupancy at a location in 3D space (Fig. 5A; Supplemental Fig. S13). We used the genomic location of XIST as the production site of XIST RNA, and we used our model with the two integrated modules to simulate XCI. The locations of the repression-enabling tethering sites for XIST were randomly chosen, and we performed 20 simulations with random selections of such tethering sites. Each simulation produced a different set of escape genes and inactivated genes. Unsurprisingly, there was a tradeoff between the percentage of the silenced chromosome and the number of experimentally determined escape genes captured by the simulations (see Supplemental Material; Supplemental Fig. S17; Berletch et al. 2015). We selected an optimal choice of tethering sites that produced balanced sensitivity and false positive rate and an overall accuracy within the inter-quartile range

of the accuracies among the 20 simulation runs with random XIST tethering sites (see Materials and Methods; Supplemental Tables S7, S8) as a representative model (Fig. 5B). All simulation runs showed a reasonable agreement with experimental data (Fig. 5C–E; Supplemental Fig. S14), in that only a small fraction of X-linked genes remained active at the steady state. Among 38 experimentally identified escape genes, the representative model captured 14 of them while maintaining a nearly 80% silencing percentage of the entire chromosome (Fig. 5E). We compared the steady state profiles of the simulated chromosome and a previously published RNA-sequencing data set containing 38 escape and 1561 silenced genes (Berletch et al. 2015), and we found that the accuracy of the model was 79.3%. Using an imposed set of tethering sites derived from experimental data (Engreitz et al. 2013) did not produce superior performance, as this model's sensitivity, specificity and accuracy were all at or below the 25th percentile of the corresponding performance measures of the models with random tethering sites (see Supplemental Material; Supplemental Figs. S16, S17), suggesting that precise tethering sites are not readily identifiable with the current model framework, possibly due to inaccurate

structural inference or the lack of epigenetic details. Nonetheless, we found that the escaped regions had a distinct radial distribution compared to the silenced regions in our simulation (Supplemental Fig. S19). Finally, we showed that, as a result of the continuous consideration of chromosome structure, silencing waves can spread across multiple chromosomal regions due to their close proximity in 3D (see an example in Fig. 5F,G). This is consistent with a previous experiment using transgene XIST that showed the possibility that gene silencing can spread through the 3D chromosome structure (Naciri et al. 2021).

In addition to simulations with static chromosome structure, we performed a simulation with dynamic chromosome structure that gradually changed from an active X chromosome configuration to an inactive X chromosome structure (Supplemental Fig. S20; Supplemental Movie S1). As expected, this simulation achieved higher coverage of silenced chromosome regions compared to models with static chromosome structure and the same set of

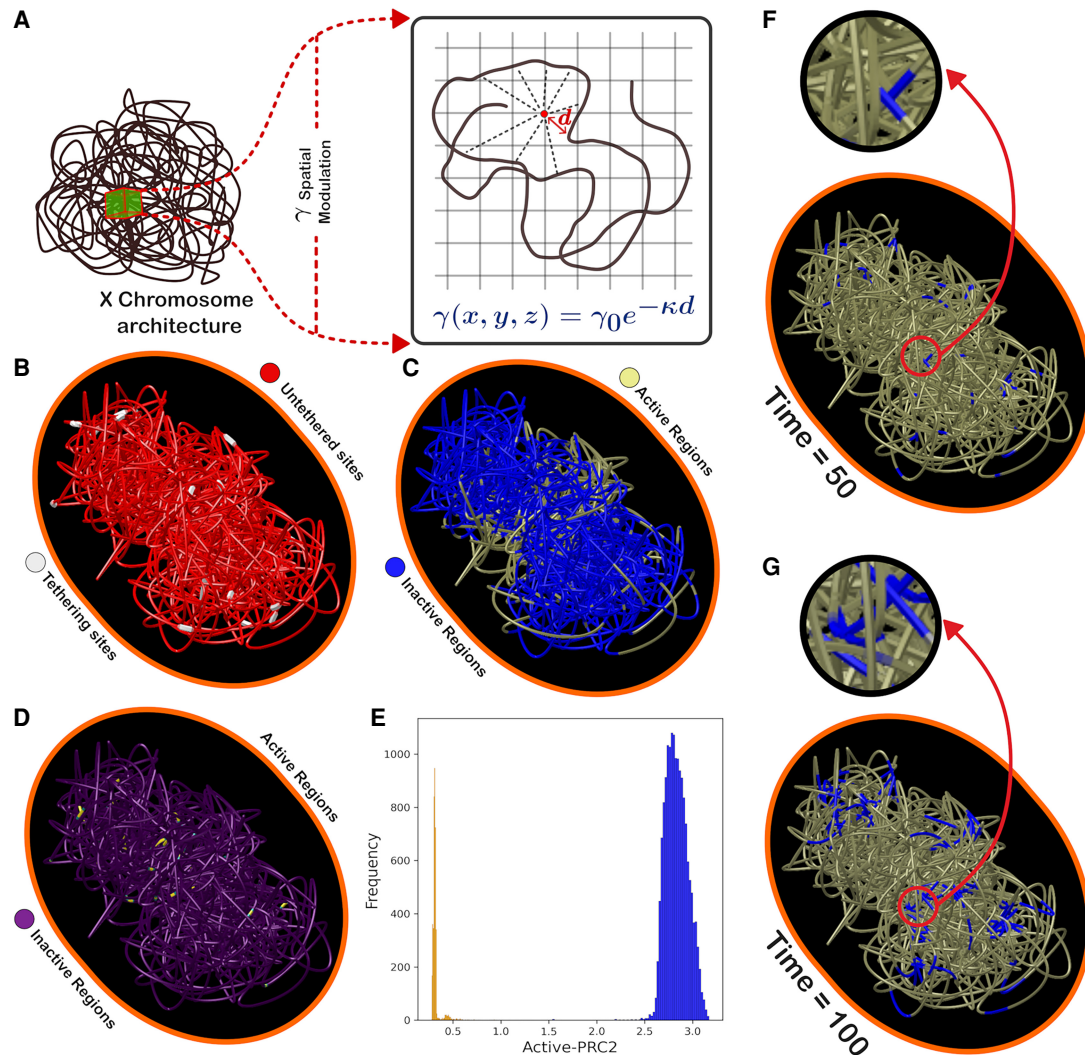


FIGURE 5. The three-dimensional model and comparison of simulated results with experimental data. (A) Schematic representation of implementing spatial dependence of the high sensitivity feedback coefficient γ associated with the process of chromosomal inactivation. (B) Spatial profile of the XIST tethering sites obtained from 3D simulation marked by white beads. (C) Spatial profile of the X chromosome inactivation obtained from simulation at time unit 500 which corresponds to ~ 2 h in physical scale (details about time and length scale estimation are provided in the Supplemental Material). (D) Visualization of the experimentally observed active and inactive genes in the X chromosome. (E) Distribution of chromosomal segments over the levels of active PRC2 in a mouse X chromosome. A total of 20,000 evenly spaced points on a simulated chromosome were used. (F,G) Demonstration of the 3D spatial spreading of the chromosomal inactivation through void space. (The detailed description of the simulation method and utilized parameter values are provided in the Supplemental Material, which includes Supplemental Table S4).

parameter values (Supplemental Table S4), resulting in a high specificity when predicting escape genes. However, the sensitivity of the dynamic chromosome model was low (Supplemental Fig. S21), with only nine escape genes captured (see Supplemental Material), reflecting the trade-off problem mentioned above. The limited performance of all 3D models is likely due to a combination of the lack of chromosomal movement at fast timescales, detailed local epigenetic factors that influence the spread of silencing wave, and more rigorous inference of chromosome structure. Nonetheless, our model produced reasonable agreement on silenced and escaped gene distributions with

experimental data using fundamental biophysical principles, and the integrative framework can be useful for future studies of XCI when more comprehensive data become available.

DISCUSSION

X chromosome inactivation (XCI) is a remarkable regulatory program for gene expression in which the transcription of a lncRNA XIST triggers gene silencing for most of the genes in a chromosome. Substantial work has been done to unravel the mechanisms of gene silencing during XCI,

but many questions remain open. In particular, it has been unclear how the spread of gene silencing is robustly triggered and regulated in a context-dependent manner. In this work, we used a mathematical model based on a continuum-based reaction-diffusion system to show that feedback loops for XIST transcription and degradation have a profound impact on both the steady state distribution of XIST and the speed to achieve the steady state. We observed that the spatial range of the XIST distribution was increased by transcription-level negative feedback (Jachowicz et al. 2022) and decreased by degradation-level negative feedback (Rodermund et al. 2021). This suggests that these feedback loops may be used to define the boundary of XIST distribution and its effect on gene silencing, and they may help to limit the XCI to a single chromosome. Previous experimental and theoretical studies without considering spatial distributions showed that negative feedback loops can accelerate the response of gene expression that steady state can be obtained rapidly (Rosenfeld et al. 2002). However, in this work, we showed that when spatial distribution of gene products is considered, transcription-level negative feedback can either speed up or slow down response, depending on the choice of other parameters. Interestingly, these functions of negative feedback loops were obtained without changing the diffusion coefficients. This may offer an alternative strategy for cells to tune molecular distributions without significant changes of the physical properties of the molecules through evolution.

The XIST-mediated gene silencing is an example of lncRNA's substoichiometric action in which a relatively low expression level of a lncRNA can trigger a large effect in gene regulation. It was previously suggested that mechanisms such as molecular condensate formation may explain this type of substoichiometric function (Unfried and Ulitsky 2022). While our model does not assume specific mechanisms for local perturbations (e.g., condensate formation or others) that lead to spread of gene silencing, it showed that systems-level positive feedback can amplify the small initial effect induced by lncRNAs and trigger bistable switches (in the case of XCI, an on-to-off switch) in a spatially sequential manner via traveling waves. This is similar to the well-known function of positive feedback for long-range communication without the need of transporting molecules over a long distance (Gelens et al. 2014). The local molecular actions of lncRNAs, such as condensates or scaffolding, can be combined with downstream positive feedback loops to give rise to large-scale effects, and this type of combination may be common among lncRNA-mediated gene regulation.

Our traveling wave model is based on the assumption of chromatin-level feedback loop between histones and enzymes that catalyze histone modification reactions. This type of feedback was widely used to explain epigenetic memory (Dodd et al. 2007). The feedback terms in our model

can also describe other sources of interactions, including those with structural-chemical cooperativity occurring at the chromosome level which may also produce feedback-like behavior (Tripathi et al. 2022). More recently, a polymer-based model showed the importance of both local attraction of epigenetic marks and global regulation of enzymes in establishing epigenetic memory with perturbed 3D chromatin structure (Owen et al. 2023). Our models show that imbalanced distributions of polycomb repressive complexes and asymmetrical silencing waves can be a mechanism for local and medium-range attraction and repulsion. The globally driven wave-pinning mechanism is consistent with the finding that global regulation of key enzymes can have significant effects on epigenetic memory.

We used a continuum-based reaction-diffusion framework for this study, and we showed the advantages of this framework in describing complex regulatory networks in a spatiotemporal model. This type of network is often difficult to model with agent-based strategies, especially when the number of molecules is large. We expect that wave-pinning and other diffusion-based mechanisms, such as the Turing pattern, can work in concert to shape the gene activity landscape on chromosomes (Paul et al. 2024). However, a key limitation of the continuum model is that the movement of small numbers of molecules and their dynamic geometries are often not described accurately. Our hybrid approach for modeling 3D chromosome structure in our model is useful for integrating continuous reaction-diffusion and discrete geometry. Furthermore, our model incorporates XIST tethering sites as the discrete points to reflect experimental observations (Table 1), providing additional flexibility for combining continuous and discrete elements in a single framework. Nonetheless, we expect that future development of modeling strategies will be necessary to describe the complex system of chromosome-level gene regulation with advantages of both continuum and agent-based methods. In particular, 3D models will benefit from more realistic, dynamic chromosome structures (e.g., those obtained from molecular dynamics simulation [Lappala et al. 2021]), more accurate quantifications of diffusion coefficients in multiple regions, and inclusion of physical and biological factors influencing the tethering sites of lncRNAs. In addition, while our model can be extended to model gene silencing mediated by transgene XIST (Lee and Jaenisch 1997; Minks et al. 2013; Naciri et al. 2021), there is a lack of autosome-wide data on escaped/silenced genes, and 3D autosome structures before and after transgene activation. These data are crucial for building realistic models that give more specific insights into factors contributing to the extent of gene silencing spread outside the context of XCI. Future experiments are therefore needed to enable our modeling framework to make predictions about gene silencing mediated by transgene XIST.

In summary, our continuum reaction-diffusion model has provided new insights into mechanisms for gene silencing spread mediated by lncRNAs, and it will help to deepen our understanding of gene regulatory networks controlling XCI in both time and space.

MATERIALS AND METHODS

Model development

We adopted a modular approach to develop our model by dividing the process of chromosomal inactivation into three main steps: (1) the spatiotemporal dynamics of XIST molecules, designated as the XIST module (Fig. 1); (2) the spatiotemporal dynamics of the inactive and active PRC2 complex, termed the chromatin (or XCI) module; and (3) the integration of the XIST and chromatin (XCI) modules, referred to as the integrated module with tethering. Throughout the main text and [Supplemental Material](#), we use these names to refer to different modules. The XIST and chromatin modules are represented by partial differential equations (PDEs) containing production, degradation, reaction, and diffusion terms. Biologically plausible parameter values were chosen to give representative results ([Supplemental Material](#)), and scanning of some parameters (e.g., feedback strengths) was performed to analyze the model behaviors systematically (e.g., Fig. 1). For the chromatin module, the diffusion coefficient of active PRC2 is assumed to be significantly lower than that of the inactive PRC2, and this is based on the assumption that active PRC2 interacts with other molecules more extensively compared to the inactive form. All simulations were performed with the no-flux boundary condition except for a few cases where the periodic boundary condition was used for further validations. The production and degradation rates are subject to feedback, which is supported by experimental evidence (see details in Results; Fig. 1A) (Rodermund et al. 2021; Jachowicz et al. 2022). For integration of the two modules, a bridging tethering module is represented by a set of conditions depending on the spatiotemporal profile of the XIST molecule that invokes the stimulation in XCI module. The tethering module introduces the stochasticity into the model arising from the limited number of transcribed

copies of XIST molecules. Unlike the first two modules that are primarily based on a continuum framework, the tethering sites of XIST are modeled as discrete points in the system to reflect previous experimental observations (Engreitz et al. 2013; Sunwoo et al. 2015). In addition to the brief description of each module provided in this section and Results, we present a detailed description in the [Supplemental Material](#).

1D Simulations

After developing and fixing various aspects of the model, we examined the basic behaviors of the system by simulating the PDEs in one dimension. We used the explicit forward Euler algorithm implemented in the FORTRAN programming language to simulate the PDEs. We discretized the space and time and then defined the Laplacian terms in each time step using a central difference formula. This formula and a complete description of numerical simulation and parameters used are included in [Supplemental Material](#). We tested the sensitivity of our main conclusions to the choice of grid size ([Supplemental Fig. S12](#)). We also implemented void space, that is, the region without the presence of chromosome by modulating the feedback coefficient γ , reflecting a biochemical component contributing to the bistability of the system. Computational details regarding the implementation of void space and spatial movement of the chromosomal region are provided in the [Supplemental Material](#). Finally, we integrated all the modules and carried out the simulation for a wider spatial region and obtained the steady heterogeneous spatial distribution which reflects the chromosomal inactivation.

3D Simulation and statistical analysis

The results of the simulations performed in one spatial dimension elicited further exploration of the system in three spatial dimensions using a realistic chromosome structure supported by experimental evidence. We carried out three-dimensional simulations using a discrete semi-empirical X chromosome structure derived from Hi-C data (Lappala et al. 2021). In addition, we implemented computational methods to simulate our continuum mathematical model over the template of a discrete three-dimensional

TABLE 1. Model types of three components and comparisons with previous studies

Component	Types of models in previous studies	Type of model in this work	New insights from the current model
XIST distribution	Discrete (Lappala et al. 2021)	Continuum	The continuum model shows the effects of feedback loops involving XIST transcription and degradation.
XIST tethering	Observed as discrete (Engreitz et al. 2013; Sunwoo et al. 2015). Not modeled.	A discrete component incorporated into a continuum model	N/A. The tethering sites are discrete points that bridge XIST concentration and gene silencing. This is consistent with experimental data.
Gene silencing dynamics	Discrete models for general epigenetic events (Dodd et al. 2007; Owen et al. 2023). No model was built for this step of X chromosome inactivation.	Continuum with a 3D chromosome structure	The continuum model provides a new explanation for robust patterning of silenced and escaped genes during chromosomal inactivation.

chromosome structure. The details of the methods are provided in the [Supplemental Material](#). Briefly, the spread of XIST molecules is described by 3D Laplacian operators. As described earlier, a parameter γ is used to describe the space-dependent feedback between XIST and chromatin and the parameter is based on the predefined 3D chromosome structure. We first used the stationary structure of the inactive X chromosome and performed the numerical simulation in 3D. The repression-enabling XIST tethering sites were randomly chosen (see [Supplemental Material](#)). A sampling of such sites was performed with 20 simulations, and we chose the simulation that produced a balanced performance in terms of both the experimentally validated escape genes (38) captured by the simulation and the known extent of repression over the mouse X chromosome reflected in the experimentally validated genes that are inactivated in XCI (Berletch et al. 2015). These two gene sets were obtained from a published RNA-sequencing data set (Berletch et al. 2015), from which we combined all mouse tissues and obtained the genes with the thresholds mentioned by the authors. Genomic coordinates were obtained for the 468 detected genes and mapped the simulated X chromosome. We analyzed the gene expression data for X-linked genes and examined the extent of agreement between the simulation results with the experimental data. In simulations, a gene is “silenced” if more than 50% of its corresponding genomic coordinates are at the inactive state at the final time point, and it is an escape gene otherwise. Detailed descriptions of conditions to filter the states of genes (ON/OFF) are provided in the [Supplemental Material](#). The accuracy of the simulation results is defined as the sum of true positives and true negatives divided by the total number of genes ([Supplemental Fig. S17](#); [Supplemental Table S7](#)). We performed the two-tailed Fisher’s exact test to quantify the statistical association between simulated results and the experimental data based on the confusion matrix (e.g., [Supplemental Table S8](#)). In addition to the 20 simulations with random sampling of XIST tethering sites, we performed a simulation with tethering sites inferred from a previous experiment (Engreitz et al. 2013). Finally, we performed a simulation with dynamic 3D chromosome structure which gradually changes from active configuration to inactive configuration (Lappala et al. 2021). Parameter values for all models are included in [Supplemental Table S9](#).

DATA DEPOSITION

Computer code for reproducing simulation results is available at the GitHub repository for this manuscript: <https://github.com/shibashispaul32/XCI>.

SUPPLEMENTAL MATERIAL

Supplemental material is available for this article.

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