Chapter 5

Summary

In this thesis, we have studied the dynamics of a single IP$_3$R cluster. That was motivated by the fundamental role that puffs occupy in the initiation of Ca^{2+} waves. The concerted action of several puffs is required to start a wave. Hence, understanding the behavior of one cluster is significant for further modeling and interpretation of experimental data.

We have analyzed the values of the Ca^{2+} concentration and of concentration gradients that occur at a cluster. We have chosen a geometry that mimics in vivo conditions, i.e. we have studied Ca^{2+} flux through a small pore in the membrane of the ER. The concentrations are 2 to 3 orders of magnitude higher than bulk concentrations. Huge gradients emerge due to the strong localization of Ca^{2+} release. This shines new light on the theoretical investigations of Ca^{2+} dynamics. The often used assumption that gating of IP$_3$ receptors is commanded by bulk concentrations cannot be held up any more. Experiments and theoretical considerations have shown that Ca^{2+} activating processes are tuned to concentration values close to the base level of about 100nM. Dissociation constants for inhibition are in the range to several μM. Thus, the large Ca^{2+} concentrations at an open cluster lead to saturation of the control mechanisms of the IP$_3$R. This restriction has to be taken into account in subsequent modeling.

We have found that release currents are proportional to the square root of the number of open channels. This holds generally and leads to novel interpretations of experimental data. We have focused on findings by Parker and collaborators about the influence of IP$_3$ on channel opening. Our results suggest that 2 IP$_3$ molecules have to bind to a receptor in a noncooperative way instead of more molecules. We have tested the impact of buffers on the concentration profiles. They have essentially no effect on the peak concentration. Consequently, the often applied experimental technique to use buffers for eliminating Ca^{2+} feedback on the channel needs a second thought. We have confirmed that coupling between

adjacent clusters is only weak, because the increase of the Ca^{2+} concentration in a distance of a few micrometers from the puff site is in the nanomolar range only. The decay of currents after termination of release could be fitted to a sum of two exponentials. This is in agreement with experimental observations. However, any interpretation with respect to gating dynamics is not possible. Especially, a conclusion toward IP_3 dependent deactivation cannot be drawn. Our simulations have revealed that facilitated diffusion plays a minor role. This contrasts previous considerations. Values of the signal mass calculated from the simulations agree very well with experimental data. Subsuming, our results serve as guidance for future modeling that has to respect the high Ca^{2+} concentrations at an open cluster.

Consequences that arise from such elevated Ca^{2+} concentrations have been presented in chapter 3. We have developed a deterministic model for Ca^{2+} release that incorporates the extreme localization of release and takes the findings of chapter 2 into account. To this aim, we have introduced a new approach to describe reactions between an immobile partner and a diffusive partner. The former has been restricted to a small area. We have performed a linear stability analysis and have shown that linear instabilities can only arise from the diffusive partner. This simplifies the procedure to a large extent and allows for analytic results. The calculations employ assumptions on the geometry of the fixed reaction partner that are applicable to a broad class of problems. Oscillations reported earlier from deterministic models with a continuous density of IP_3R vanish when we insert the realistic concentrations from chapter 2. We have found a monostable regime. We have shown in addition that the oscillations that occur in our model do not correspond to the experimentally observed ones. On the one hand, the oscillatory regime is too small and occurs at unphysiological parameter values. On the other hand, the amplitudes of the oscillations do not correspond to measured values.

These results have finally proved that deterministic models based solely on IP_3 activation, Ca^{2+} activation and Ca^{2+} inhibition do not capture the correct mechanisms of IP_3 mediated Ca^{2+} release. Our findings demonstrate the necessity of stochastic approaches in modeling of intracellular Ca^{2+} dynamics. Fluctuations are crucial for Ca^{2+} oscillations and have to be taken seriously in any future investigation.

Consequently, we have derived a master equation and two corresponding Fokker-Planck equations for the dynamics of an IP_3R cluster. We have employed a state scheme of the IP_3 receptor that exceeds the one normally used. It incorporates fluctuations in the Ca^{2+} activation processes. In contrast to previous works that couple the gating dynamics to the bulk concentration of Ca^{2+}, we have used the

results of chapter 2 and 3 to implement realistic parameter values. We have calculated the stochastic part of the puff frequency for all three models from a first passage process. It is driven by fluctuations, so that the noise in the Ca^{2+} activation must not be neglected as done earlier. On the time scale of Ca^{2+} activation, the Ca^{2+} inhibiting processes may be considered constant in time. Therefore, we have computed the mean first passage time with a one dimensional model. This could be done analytically for the master equation and the Ω expansion. For the parameters chosen, we have discovered that the stochastic part of the period is not accompanied by channel opening which happens later. This is an counterintuitive result, but gave us certainty that we have indeed calculated the stochastic fraction of puff frequencies. If channel opening had already occurred during the time for the escape, an interpretation of the mean fist passage time as the stochastic fraction of the puff frequency would have been doubtful.

Estimating the stochastic part is a first step toward a comprehending analysis of puff frequencies and hence wave nucleation. A calculation of the remaining fraction requires different techniques. As soon as one channel opens, transients as in figure 3.8 occur and essentially influence the dynamics of the IP_3 receptor. Moreover, the time scale separation between Ca^{2+} inhibition and Ca^{2+} activation might not hold any more. This amounts to a detailed study of equation (4.1). The two dimensional escape process represents the natural extension of this work and will be investigated more closely in the future.

This thesis has provided clear evidence that intracellular Ca^{2+} is a truly stochastic medium. As a pattern forming system, it offers intriguing properties. Fundamental stochastic events (puffs) and deterministic phenomena (waves) can be observed at the same time. The parameters that shape the Ca^{2+} dynamics are easily controlled. This renders intracellular Ca^{2+} a model system for non equilibrium physics. The field has the potential that many studies like this will follow.