

## Perspective

# Ca<sup>2+</sup> Sparks in Frog Skeletal Muscle: Generation by One, Some, or Many SR Ca<sup>2+</sup> Release Channels?

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Ca<sup>2+</sup> sparks were first detected as spontaneous, highly localized elevations of Ca<sup>2+</sup> indicator fluorescence in confocal microscope images of rat cardiac myocytes studied under resting conditions (Cheng et al., 1993). It was immediately suggested that these events are likely to reflect the localized release of Ca<sup>2+</sup> from a small cluster of sarcoplasmic reticulum (SR) Ca<sup>2+</sup> release channels or perhaps even from a single SR channel (Cheng et al., 1993). Ca<sup>2+</sup> sparks were found to occur at increased rates during small depolarizations of rat cardiac myocytes, leading to the concept that the macroscopic [Ca<sup>2+</sup>] transient during larger depolarizations of cardiac myocytes might be due to the spatio-temporal summation of such events occurring at high frequencies throughout the myocyte (Cannell et al., 1993, 1995; López-López et al., 1994).

Ca<sup>2+</sup> sparks also occur in frog skeletal muscle fibers, both “spontaneously” in resting fibers (Klein et al., 1996) and at higher frequencies during small depolarizations that produce relatively low levels of voltage activation of Ca<sup>2+</sup> release (Tsugorka et al., 1995; Klein et al., 1996). As in cardiac myocytes, during large depolarizations of frog skeletal muscle fibers such events could occur at much higher rates and spatio-temporally summate to compose the macroscopic [Ca<sup>2+</sup>] transient. The present perspective focuses on Ca<sup>2+</sup> sparks in frog skeletal muscle and the possible SR Ca<sup>2+</sup> release channel activity that underlies the generation of the observed sparks. A central unresolved issue is whether a spark is generated by a single channel, by a small cluster of a few channels, or by a larger strip of many contiguous channels in the SR junctional face membrane.

### Observations and Interpretations

*Spark fluorescence and the underlying time course of SR Ca<sup>2+</sup> release.* Before discussing the number of channels responsible for generating a Ca<sup>2+</sup> spark, it is important to first consider what information the spark provides concerning the underlying time course of SR Ca<sup>2+</sup> release. The signal actually monitored in studies of Ca<sup>2+</sup> sparks is the change in fluorescence of a calcium indicator,

generally fluo-3, within the confocal volume in a confocal line scan image. The observed time course of fluorescence at the spatial center of a spark can be interpreted using a qualitative approach that provides a general perspective on the possible time course of the underlying Ca<sup>2+</sup> release and serves as a starting point for the present considerations. During the rising phase of a spark, the concentration of Ca<sup>2+</sup>-fluo-3 must be increasing in the confocal volume. This indicates that Ca<sup>2+</sup> entry into the confocal volume must exceed the net effect of Ca<sup>2+</sup> “removal” by binding and diffusion out of the confocal volume. Thus, during the rising phase of a spark, Ca<sup>2+</sup> ions are being released from the channel or channels responsible for generating the spark (Fig. 1 A). In contrast, during the falling phase of fluorescence in a spark, there is a net fall of Ca<sup>2+</sup>-fluo-3 in the confocal volume, indicating that Ca<sup>2+</sup> binding and Ca<sup>2+</sup> diffusion out of the confocal volume exceed Ca<sup>2+</sup> entry. Since the diffusion and Ca<sup>2+</sup> binding properties of the myofibril are unlikely to change significantly during the spark, the declining phase of a spark must correspond to a period during which Ca<sup>2+</sup> release occurs at a much lower rate than during the rising phase. In the extreme case, Ca<sup>2+</sup> release could occur at an approximately constant rate during the rising phase of the spark, and then stop completely during the falling phase (Fig. 1, B–D).

*Ca<sup>2+</sup> release turns on and off abruptly at the start and peak of a spark.* Our recent studies using relatively high speed (63 μs per line) line scan confocal imaging of Ca<sup>2+</sup> sparks in frog skeletal muscle provide a 30-fold increase in the available temporal resolution of spark time course and give results that are not inconsistent with the extreme interpretation in Fig. 1. These studies demonstrate a very abrupt transition from rising to falling fluorescence at the peak of the spark, as shown by the theoretical time course in Fig. 1 A, which provides a good representation of most aspects of the observed sparks (Lacampagne et al., 1999). The sharp peak in the observed spark time courses indicates a large and abrupt decrease in the rate of Ca<sup>2+</sup> release rate at the peak of the spark. From our results, it does not seem

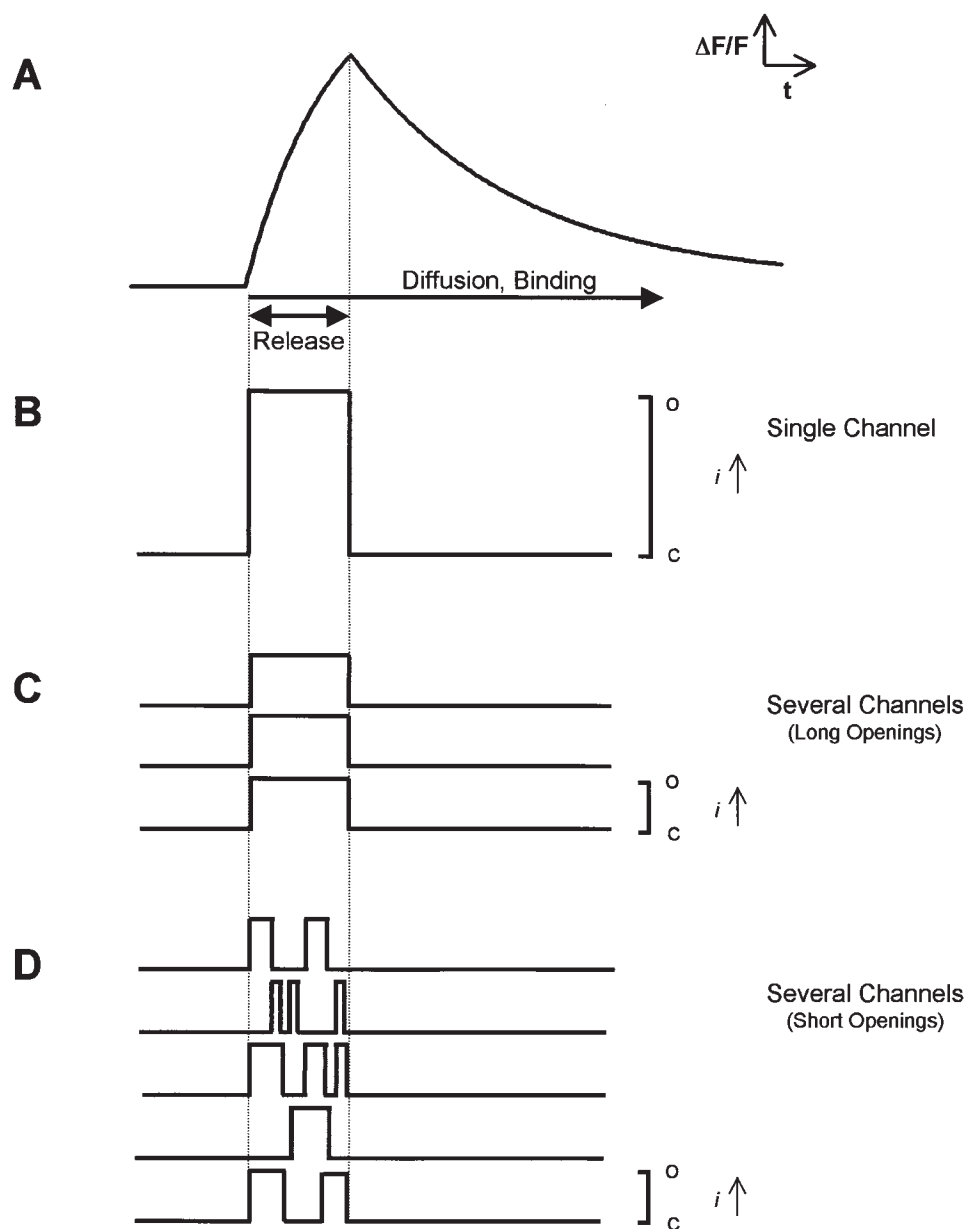


FIGURE 1. Hypothetical alternative patterns of SR  $\text{Ca}^{2+}$  release channel activity that could underlie the  $\text{Ca}^{2+}$  sparks observed in frog skeletal muscle. (A) Theoretical function representing the relative change in fluorescence ( $\Delta F/F$ ) as a function of time ( $t$ ) in a typical  $\text{Ca}^{2+}$  spark. The line was generated by the function  $\Delta F/F = 0$  before the first vertical dashed line,  $\Delta F/F = A(1 - \exp[-k_1(t - d_1)])$  between the two vertical dashed lines, and  $\Delta F/F = A\{1 - \exp[-k_1(d_2 - d_1)] \exp[-k_2(t - d_2)] + C\}$  after the second vertical dashed line. This function, which has discontinuous derivatives at both its start and peak, systematically closely followed almost all aspects of the time course of  $\text{Ca}^{2+}$  sparks recorded experimentally with video rate recording (Lacampagne et al., 1999). The parameter values obtained from a least squares fit of this function to a  $\text{Ca}^{2+}$  spark ( $k_1 = 340 \text{ s}^{-1}$ ,  $k_2 = 214 \text{ s}^{-1}$ , and  $d_2 - d_1 = 3.3 \text{ ms}$ ) were used to generate the theoretical record shown here. (B–D) Three alternative hypothetical classes of activity patterns, either of an individual SR  $\text{Ca}^{2+}$  release channel (B) or of a group of three (C) or five (D) channels, that could underlie the observed  $\text{Ca}^{2+}$  spark time course. Each individual record represents the time course of current ( $i$ ) through a single channel, with  $c$  and  $o$  denoting the closed and open state of the channel. Note that the time course of total  $\text{Ca}^{2+}$  efflux is approximately the same for each channel activity pattern in B–D.

implausible that release could turn off completely at the peak of a spark. In this case, the rise time of the spark would correspond to the total time that the channel or group of channels generating the spark were open, and the declining phase would be a time during which the release rate were zero. Since the high time resolution studies also demonstrate a rather abrupt transition to a high rate of rise of fluorescence at the start of a spark, the level of  $\text{Ca}^{2+}$  release activity underlying the spark also seems to achieve a near maximal rate early in the spark rising phase. In this case, the net level of overall release channel activity in a spark would rapidly jump from zero to a constant rate early in the rising phase, remain approximately constant throughout the rising phase, and then rapidly fall off to zero at

the peak of the spark. As indicated diagrammatically in Fig. 1, this interpretation could correspond to a single channel open for the entire rising phase of the spark (B), multiple channels, each of which remain open throughout the rising phase of the spark (C), or multiple channels that open and close asynchronously and repeatedly during the rising phase of the spark but close within a short interval at the time of peak of the spark (D). In a more general interpretation, the rise time of a spark provides a lower limit for the open time of channels responsible for generating the spark since some channel(s) might open or remain open during the declining phase, even though the rate of release during the declining phase of the spark must have been markedly less than during the rising phase (not illustrated).

The above types of interpretation can be made quantitative through the use of detailed modelling of  $\text{Ca}^{2+}$  binding and diffusion in a fiber after release from a channel or group of channels (e.g., Pratusевич and Balke, 1996). These quantitative models indicate that release could abruptly turn on and off at the start and peak of the observed sparks.

*Properties of individual sparks are not obviously incompatible with  $\text{Ca}^{2+}$  release from a single channel.* The  $\text{Ca}^{2+}$  sparks detected in a frog skeletal muscle fiber have an average rise time of 4.6 ms and an average decay time constant of 8.6 ms (Lacampagne et al., 1999) and an average spatial full width at half max of  $\sim 1.4\text{--}1.5\ \mu\text{m}$  (Lacampagne et al., 1996). The peak change in relative fluorescence ( $\Delta F/F$ ) of the larger amplitude events, which are most likely to represent events arising spatially closest to the scan line, is  $\sim 1\text{--}2$ . These properties, together with the diffusion and binding properties of the fiber, provide an indication of the amount of  $\text{Ca}^{2+}$  released by the channel or channels that generate the spark. It was already pointed out in early reports on cardiac  $\text{Ca}^{2+}$  sparks that rough approximations of the amount of  $\text{Ca}^{2+}$  released in a spark were not obviously incompatible with channel open times and  $\text{Ca}^{2+}$  flux rates of single SR  $\text{Ca}^{2+}$  channels in bilayers (Cheng et al., 1993). Using detailed models of the sarcomeric distribution of myoplasmic  $\text{Ca}^{2+}$  binding sites together with the diffusion properties of  $\text{Ca}^{2+}$  and fluo-3, it is possible to calculate the spatio-temporal distribution of  $\text{Ca}^{2+}$ –fluo-3 and the resulting  $\text{Ca}^{2+}$  spark that would be produced by an assumed time course of  $\text{Ca}^{2+}$  release from a point source corresponding to the channel or group of channels generating the spark. These modelling calculations, using reasonable values for the various model parameters and reasonable values for single channel current and channel open time (e.g., 1–2 pA of current for  $\sim 10$  ms) result in theoretical  $\text{Ca}^{2+}$  sparks that are not obviously incompatible with the observed sparks (Pratusевич and Balke, 1996; Jiang et al., 1998). Thus, based on such calculations alone, it is not necessary to exclude the possibility that a  $\text{Ca}^{2+}$  spark could be generated by the  $\text{Ca}^{2+}$  released during the opening of a single SR  $\text{Ca}^{2+}$  release channel. However, it should be noted that this finding does not imply that  $\text{Ca}^{2+}$  sparks are in fact generated by the activity of a single SR  $\text{Ca}^{2+}$  release channel, but only establishes that the experimentally observed sparks are not obviously quantitatively inconsistent with the possibility of spark generation by a single channel.

*Spark frequency increases during activation, but spark properties remain constant.* The “spontaneous” sparks observed in frog fibers appear to be ligand-gated events triggered by calcium-induced calcium release (CICR) since the frequency of spontaneous events increases with increased myoplasmic [ $\text{Ca}^{2+}$ ] and in the presence of caffeine

(Klein et al., 1996), and decreases with increased myoplasmic [ $\text{Mg}^{2+}$ ] (Lacampagne et al., 1998), all hallmarks of CICR. The sparks initiated by fiber depolarization are voltage-activated events, presumably triggered by activation of voltage sensors (Schneider and Chandler, 1973), the dihydropyridine receptors (Tanabe et al., 1987) in the transverse tubule (TT) membrane of the fibers. One of the salient features of  $\text{Ca}^{2+}$  release by both ligand- and voltage-activated  $\text{Ca}^{2+}$  sparks in frog skeletal muscle fibers is that the overall level of calcium release appears to be graded by variations of the frequency of occurrence of  $\text{Ca}^{2+}$  sparks, but that the individual sparks themselves have similar average properties despite marked differences in their frequency of occurrence. For example, with protocols that use relatively brief repriming of chronically depolarized fibers, the frequency of occurrence of voltage-activated events can be modulated by both the extent of repriming and by the membrane potential of the test depolarization used to activate events after repriming, but neither of these parameters appears to affect the average amplitude or average rise time of the detected events (Lacampagne et al., 1996; Klein et al., 1997). Lowering myoplasmic free [ $\text{Mg}^{2+}$ ] increases the spontaneous frequency of ligand-gated events, but does not alter the average properties of the individual events (Lacampagne et al., 1998). Thus, the average properties of the individual events appear to be quite constant despite relatively large changes in their frequency.

An interesting implication of these observations is that the opening rates, but not the closing rates, of the channel(s) underlying the spark appear to be modulated during release activation. The opening rate of the channel(s) that initiate the spark must be increased by the voltage or ligand activation that caused the observed increases in spark frequency since spark frequency directly reflects the rate of opening of the channels that initiate the sparks. In contrast, the overall open time of the channels generating the spark, and thus the effective rate of channel closing, appears to be unchanged. The mean amplitude and rise time of the sparks should change in parallel with any changes in the net open time of the channels generating the spark (Fig. 1), but no such changes were observed. Net channel open time could change without a parallel change in the observed spark rise time only for the case of asynchronous opening of multiple channels during the rising phase of a spark (Fig. 1 D), but the spark amplitude would then still vary with changes in the net open time of the channels underlying the spark. However, no changes in mean spark amplitude were observed under the conditions in which spark frequency was markedly increased. Thus, channel closing rates do not seem to be altered under the conditions used for voltage or ligand activation of the sparks.

In interpreting these results, it is important to bear in mind several technical issues. In the case of both voltage- and ligand-activated sparks, only events occurring at relatively low frequency were examined in order to allow relatively unambiguous identification of individual events. Even in the case of relatively low-event frequencies, there are two other technical issues to consider. First, only events above an arbitrarily selected low-amplitude cut-off were selected for analysis. This cut-off was imposed to avoid including possible noise as events in the analysis of  $\text{Ca}^{2+}$  sparks. Second, the amplitude of each observed event is very sensitive to the site of origin of the  $\text{Ca}^{2+}$  release source relative to the spatial location of the confocal scan line. Since these relative locations are completely random in our studies, a variation of amplitude is introduced simply by the range of release site locations relative to the scan position. However, despite these two limitations, it would seem that if the actual average amplitude of the sparks did increase with increased frequency of occurrence, then the average amplitude of the sparks detected above the arbitrary cut-off amplitude and arising at random locations relative to the scan line should also have increased. Yet no significant changes in spark amplitudes were detected under these conditions of different spark frequencies. Furthermore, mean spark rise time, which represents the effective duration of  $\text{Ca}^{2+}$  release and is less sensitive to differences in spark location relative to the scan line (Pratusevich and Balke, 1996), was also constant under the conditions of different spark frequencies.

*Possible regulation of channel opening or  $\text{Ca}^{2+}$  release during a spark.* By analyzing sparks that occur repetitively at particular locations at much higher rates than the average frequency over the entire fiber, the limitations of variable spark origin and arbitrary cut-off of event amplitudes discussed in the preceding paragraph can to a large extent be overcome. Such higher frequency events occur spontaneously in cardiac myocytes (Parker and Wier, 1997) and both spontaneously and during depolarization in frog skeletal muscle fibers (Klein et al., 1999) and appear to represent a repetitive mode of spark activation. If these repetitive events are generated by the repeated opening of a given channel or small cluster of channels (Klein et al., 1999), they would all arise at the same spatial site within the fiber. Thus, variation in spark amplitude due to variation in the site of origin relative to the scan line would not be a factor in the relative amplitude of the individual events within a given repetitive train. Analysis of events in such trains indicates an unanticipated lack of smaller events, even when objective procedures are employed to evaluate possible occurrence of unidentified events between identified events (Klein et al., 1999). Examination of the records of repetitive events from cardiac myocytes (Parker and Wier, 1997) also indicates a relative lack of smaller amplitude events. If the

sparks in a repetitive train were generated by the opening of a single channel having a single or multiple exponential open time distribution and if the spark amplitude were directly related to the channel open time, then smaller events would actually be expected to occur more frequently in the train than larger events. Thus, if a single channel is responsible for generating the repetitive sparks, the observed paucity of smaller amplitude events in repetitive trains would indicate the possibility of some sort of regulation of the amount of  $\text{Ca}^{2+}$  released by the single channel generating the spark.

Data from our recent high time resolution studies of nonrepetitive  $\text{Ca}^{2+}$  sparks may also be consistent with nonregulation of the amount of  $\text{Ca}^{2+}$  released in a spark. In these experiments, we have observed an inverse relationship between the mean rate of rise of fluorescence in groups of sparks having similar rise times and the mean spark rise time, resulting in a constant mean amplitude in groups of sparks having different rise times (Lacampagne et al., 1999). These observations are consistent with the possibility of negative feedback between the local elevation of  $[\text{Ca}^{2+}]$  and the continuation of  $\text{Ca}^{2+}$  release from the channel or channels responsible for generating the spark. Given the complexity of regulation of individual SR  $\text{Ca}^{2+}$  release channels (Meissner, 1994), it is not inconceivable that such feedback regulation could occur with a single channel generating a spark. Alternatively, a relatively constant amount of  $\text{Ca}^{2+}$  release in different events in a repetitive train could also conceivably occur if multiple SR channels were responsible for generating a spark.

*Are two types of sparks activated during fiber depolarization?* In our original report of the existence of ligand- and voltage-activated sparks in skeletal muscle, the observed amplitude distributions of the sparks initiated by these two types of mechanisms were different (Klein et al., 1996). The spontaneous (i.e., ligand-gated) sparks appeared to correspond to a single population of events, whereas the voltage-activated sparks appeared to correspond to one population of events having the same amplitude distribution as the spontaneous events together with another population of events having an amplitude distribution corresponding to approximately twice that of the spontaneous events. The smaller amplitude voltage-activated events were attributed to the opening of a single SR  $\text{Ca}^{2+}$  channel directly by interaction with the voltage sensor. The larger amplitude events were attributed to opening of two channels, one activated directly by the voltage sensor and a second activated by CICR due to the locally elevated  $[\text{Ca}^{2+}]$  in the immediate neighborhood of the voltage-activated channel. In this case, at least two channels would have to be involved in generating the population of larger voltage-activated  $\text{Ca}^{2+}$  sparks. However, in subsequent studies in our laboratory, we have found no obvious differences in the

mean amplitudes of ligand- and voltage-activated sparks in different fibers. In our initial study (Klein et al., 1996), we employed levels of activation that resulted in the occurrence of relatively large numbers of sparks at relatively high frequencies since we were searching for evidence for the existence of sparks. Although this strategy may have been appropriate for an initial demonstration of the existence of these events, it was not ideal for the detailed characterization of the properties of individual events due to the possibility of events randomly overlapping in space and time. Although the possibility of chance overlap of random independent events was discounted in our original report, our estimate of the probability of random overlap did not include possibly overlapped events in the estimate of the total event rate used for the calculation. Based on our subsequent experience, it now seems possible that the event rate during the depolarizations in our initial study may have been too high to permit accurate determination of the properties of isolated individual sparks. Thus, the question of the relative amplitudes of ligand- and voltage-activated events in the same fibers should probably be reexamined using lower event rates during fiber depolarization.

Studies using relatively small depolarizations together with pharmacological approaches to selectively modulate a possible CICR component of voltage-activated sparks in frog skeletal muscle fibers have supported the concept of smaller events during depolarization being directly activated by the voltage sensor and larger events having an additional contribution due to local CICR (Shirokova and Ríos, 1997). It will be important to rule out various alternative interpretations, such as possible modulation of channel open time and/or channel conductance or of SR  $\text{Ca}^{2+}$  content due to the pharmacological agent in these or other studies using pharmacological interventions in which changes in average properties of individual sparks are detected. Finally, it is possible that RyR1 and RyR3, the two different mammalian skeletal muscle ryanodine receptor (RyR) isoforms that both have homologues expressed in frog skeletal muscle, may play different roles or release different amounts of  $\text{Ca}^{2+}$  in the generation of  $\text{Ca}^{2+}$  sparks. The failure to detect  $\text{Ca}^{2+}$  sparks in adult rat skeletal muscle (Shirokova et al., 1998), which expresses only RyR1, and the observation that expression of RyR3 but not RyR1 causes the appearance of  $\text{Ca}^{2+}$  sparks in myotubes from a myogenic cell line lacking any RyR expression (Ward et al., 1999) point to different properties of these two isoforms and to the possibility that one or more RyR3  $\text{Ca}^{2+}$  channels or their frog homologue may be required for the production of a detectable  $\text{Ca}^{2+}$  spark in skeletal muscle. If coordinated activity in both types of RyR channels is involved in the generation of some sparks in frog muscle fibers, then at least these sparks must require two or more channels.

*Could a single SR  $\text{Ca}^{2+}$  channel trigger a multichannel release unit?* Under conditions of low average rates of occurrence of  $\text{Ca}^{2+}$  sparks as used in our recent experiments, it seems likely that each voltage-activated spark is initiated by the activation of the SR channel controlled by a single TT voltage sensor and that each ligand-activated event is initiated by the opening of a single SR  $\text{Ca}^{2+}$  release channel by CICR. Thus, if a spark involves the opening of multiple SR  $\text{Ca}^{2+}$  release channels, the single channel that opens to initiate the spark must activate one or more neighboring channels, presumably by CICR. These channels could in turn activate additional neighboring channels, which in principle could continue until all the SR channels along an entire region of continuous TT-SR junctional couplings were activated. For the typical relatively brief sparks having a rising phase of a few milliseconds duration, it might be imagined that the propagation of activation would have to occur rapidly at the start of the rising phase, and that the rising phase would end as the channels close in near synchrony, possibly by calcium-dependent inactivation. This general type of propagated activation scheme involving many SR  $\text{Ca}^{2+}$  channels has been simulated using a model of possible local  $\text{Ca}^{2+}$  signalling within the TT-SR junctional region (Stern et al., 1997). Thus, the possibility that activity of many SR channels coupled by CICR underlies a spark is not theoretically inconsistent with initiation of the spark by activation of a single channel.

In considering the possibility of propagation of activation by CICR from the SR channel to a channel along a strip of TT-SR junctional contact, it is relevant to reiterate that lowering myoplasmic  $[\text{Mg}^{2+}]$ , which decreases the inhibitory influence of  $\text{Mg}^{2+}$  on CICR, increases the frequency of spontaneous sparks but does not alter the mean amplitude or mean rise time of the sparks (Lacampagne et al., 1998). These observations indicate that the number of SR channels contributing to a spark does not seem to be influenced by  $[\text{Mg}^{2+}]$ . Thus, if propagation of activation from the SR channel to a channel along the junctional region by local CICR is responsible for generating the spark, the number of channels activated by propagation along the TT-SR junctional strip does not seem to be significantly affected by alterations in  $\text{Mg}^{2+}$  inhibition of CICR. One explanation could be that the safety factor for propagation is sufficiently high that all SR channels in the strip are activated at all levels of  $[\text{Mg}^{2+}]$  tested so that activation was essentially "all or none" at the level of an individual junctional strip. Alternatively, propagation along the junctional strip may in fact not occur, in which case each spontaneous spark would be generated by the opening of only a single SR channel by CICR.

*Could a single SR  $\text{Ca}^{2+}$  channel maintain prolonged release in many other channels?* We have observed that applica-

tion of Imperatoxin A (IpTx<sub>a</sub>) to a permeabilized frog muscle fiber causes the appearance of prolonged Ca<sup>2+</sup> sparks having durations of several hundred milliseconds or longer (Shtifman et al., 1999). Since IpTx<sub>a</sub> is known to produce similarly prolonged subconductance openings (about one-third conductance of normal channel opening) of individual SR Ca<sup>2+</sup> release channels incorporated in lipid bilayers (Tripathy et al., 1998), possibly by acting as an analogue of the 2–3 cytoplasmic loop of the TT dihydropyridine receptor/voltage sensor, it seems likely that the prolonged sparks observed in the presence of IpTx<sub>a</sub> were generated by the prolonged opening of a single SR channel to a subconductance state. The very long duration sparks initiated by IpTx<sub>a</sub> were also smaller in amplitude than normal short-duration events observed in the same fibers. These observations raise the interesting question of whether the prolonged toxin-induced opening of a single SR channel could maintain the opening of many other SR channels without the other channels inactivating. Alternatively, the prolonged toxin-induced sparks could be readily explained if toxin-induced sparks are generated by the prolonged opening of only the single channel interacting with the toxin. In this case, the observation that the amplitude of the toxin-induced event is smaller than that of a brief toxin-independent spark, together with the fact that IpTx<sub>a</sub> produces a subconductance state when applied to isolated SR channels, could indicate that the normal sparks observed in the absence of toxin could also be generated by the opening of a single SR Ca<sup>2+</sup> release channel, but to the full

conductance state and only for a few milliseconds.

### Conclusion

Based on the various considerations presented above, it does not appear that we can yet exclude the possibilities that either one or many channels are involved in the generation of a Ca<sup>2+</sup> spark. If a single channel is responsible for generating a spark, the single channel must have appropriate feedback regulation so as to account for the reproducible spark amplitude and relative lack of small events during the repetitive spark gating mode observed at occasional triads. On the other hand, the very prolonged small amplitude sparks observed in the presence of IpTx<sub>a</sub> are readily explained on the basis of prolonged subconductance opening of a single toxin-bound channel. If many channels are involved in the normal, short duration voltage- or ligand-activated spark, they would have to open and close in close synchrony, or burst over the same few milliseconds time interval to account for the abrupt start and peak of the observed spark time course. If many channels are involved in a short spark, it would also seem to be necessary for a single open channel to have the capability of maintaining long duration opening of at least some of the other channels involved in the short spark to account for the long duration events produced by IpTx<sub>a</sub>. It will be an interesting challenge to try to resolve these still viable important alternative possibilities as to the channel activity pattern underlying the Ca<sup>2+</sup> sparks observed in frog skeletal muscle.

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