for CH_3 binding to the Ni site is presented in this issue of *Science* (*3*).

To complete the biological reaction, CoASH reacts with the intermediate shown in the figure to form the thioester CoAS-C(O)Me (reaction **1**). The mechanism by which this C–C bond-forming process occurs is still under scrutiny (*16, 17*). The simplest explanation would involve the formation of an intermediate acyl [M-$C(O)CH_3$] (M = Fe or Ni) species, and evidence does indeed point to an acyl intermediate in reaction **1**. It is not clear whether an Fe^- or Ni^- acyl intermediate is involved. With synthetic organometallic systems, CO insertion into M–CH_3 bonds, to form M–$C(O)CH_3$, is one of the most fundamental reaction types (*1*). These reactions generally occur at mononuclear (single metal) sites that contain electron-accepting supporting ligands such as PR_3, C_5H_5 (Cp), or CO. Acyl formation occurs by means of methyl migration to the carbon of an adjacent CO. The best known example of this is the Monsanto acetic acid process (reaction **2**), the catalyst of which is shown in the figure. An obvious distinction between the biological system CODH and synthetic catalysts is the absence of electron-accepting PR_3, Cp, or CO ligands, because they are not biologically available. Instead, nature is limited to ligands L-S, N, and O, which are not typically found to encourage acetate formation. A few rare examples of synthetic models for the CODH Ni site containing biologically relevant ligands (S, N, or O) have been reported, however (*18–21*). Mononuclear (S, N)-ligated Ni–CH_3 species have also been shown (*17, 18, 21*) to react with CO to form acyl complexes, and then to convert to thioesters upon addition of thiols. This is directly relevant to the proposed pathway of acetyl-CoA synthase reactivity. Complexes of Ni(I)–CO with biologically relevant ligands are also known (*18, 20*).

The question that remains concerns the role of the Fe_4S_4 cluster in CODH. Given the synthetic model reactions described above, it would appear that Ni is capable of undertaking the entire CODH reaction scheme without the aid of an Fe_4S_4 cluster. In fact, synthetic Fe_4S_4 clusters are unstable in the presence of CO under reducing conditions (*22*). It has been proposed (*16, 17*) that the more oxidized Fe_4S_4 cluster serves as a CO binding site, and that CO insertion, involving the Fe_4S_4–CO intermediate shown in the figure, is promoted by redox changes at the cluster site. This has yet to be synthetically modeled. Synthetic models have shown, however, that in order for thioester formation to take place at a Ni center, the Ni ion must be reduced by $2e^-$ [from Ni(II) to Ni(0)] (*17*). It is therefore possible that the Fe_4S_4 cluster in CODH serves to facilitate the removal of these two electrons, a step that appears to be critical to the stability of the Ni site. The reconciliation of the mononuclear pathway of acyl formation observed with synthetic systems, and the binuclear pathway proposed to occur with CODH (*16, 17*), awaits further study.

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Calcium Sparks in Vascular Smooth Muscle: Relaxation Regulators

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Many smooth muscle cells periodically exhibit spontaneous transient outward (hyperpolarizing) currents, or STOCs (*1*). Each of these events results from the opening of 10 to 100 potassium-selective channels, triggered simultaneously by a rise in cytoplasmic calcium concentration ($[Ca^{2+}]$). Because these STOCs can be suppressed by agents that interfere with the release of Ca^{2+} from intracellular stores, the triggering Ca^{2+} for the STOCs has been presumed to come from inside the cell (*1, 2*). It has so far been impossible to detect the rise in Ca^{2+} that activates the STOCs—presumably because the Ca^{2+} increase is highly localized and brief, and thus invisible in whole-cell Ca^{2+} recordings or Ca^{2+} images with low time resolution. Now, Nelson and co-workers report in this issue of *Science* the first sighting of these local increases in Ca^{2+} in the cytoplasm of single smooth muscle cells (*3*). This first glimpse of these "Ca^{2+} sparks" is exciting for understanding how STOCs are generated, but perhaps even more exciting is the demonstration that sparks are quite likely responsible for a specific cell function—a vasodilatory influence on small cerebral arteries.

The Ca^{2+} sparks of smooth muscle are not quite the same as those in cardiac muscle, the tissue in which sparks were first reported (*4*). The Ca^{2+} sparks in both muscle types do have a similar duration (~100 ms), magnitude (a few hundred nM), and spatial extent (2 μm diameter at half-maximal $[Ca^{2+}]$). In both tissues, the Ca^{2+} sparks arise from the opening of one or several ryanodine receptors and reflect the activation of an elementary Ca^{2+}-release unit. In cardiac muscle, the sparks are recruited throughout the cell to produce the global rise in $[Ca^{2+}]$ that causes the synchronous activation of the contractile system and the consequent ejection of blood from the heart (*5*). However, the Ca^{2+} sparks in smooth muscle are generated in isolation principally near the cell surface, presumably reflecting the fact that in smooth muscle the sarcoplasmic reticulum (SR), enriched in ryanodine receptors, is near the cell surface (*6*). These ryanodine-sensitive release units are thus perfectly positioned to receive signals from the plasma membrane and to send signals in the form of localized Ca^{2+} increases. In cardiac muscle, the ryanodine receptor amplifies Ca^{2+} signals arising from the plasma membrane. The studies of Nelson and co-workers, however, show

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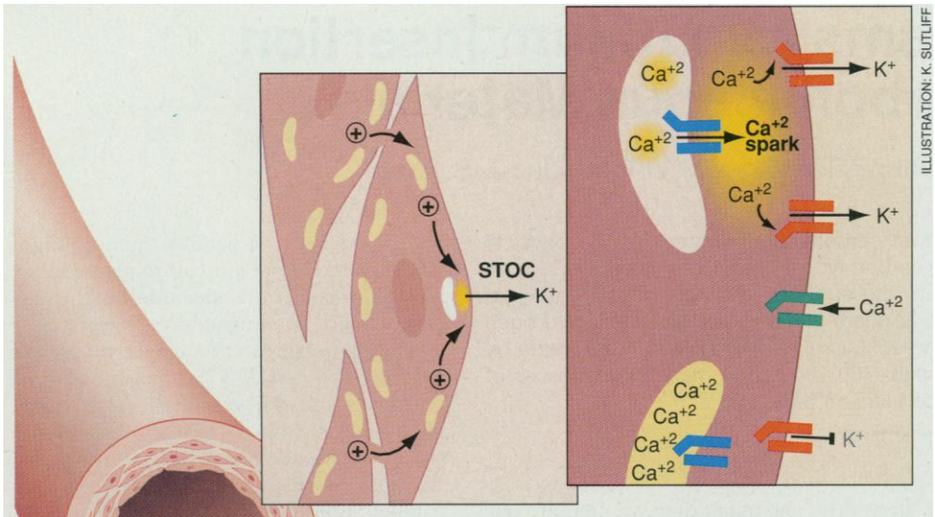

How Ca^{2+} sparks might regulate vasodilation. Local release of Ca^{2+} from internal stores activates K^+ channels, and the resulting K^+ efflux causes membrane hyperpolarization. This decrease in membrane potential causes voltage-sensitive Ca^{2+} channels to close, thereby causing a drop in cytoplasmic Ca^{2+} and diminished smooth muscle contraction. A single Ca^{2+} spark affects the activity of several smooth muscle cells due to electrical coupling of cells.

that the job of the ryanodine receptor is different in smooth muscle, where receptors on internal Ca^{2+} stores "instruct" channels on the plasma membrane what to do.

Nelson *et al.* present three lines of evidence linking Ca^{2+} sparks and STOCs. (i) The average time course of both events is very similar; (ii) both events originate on or near the surface of the cell; and (iii) agents that interfere with Ca^{2+} sequestration by internal stores inhibit, at similar concentrations, the appearance of both Ca^{2+} sparks and STOCs. Future studies will need to demonstate that the appearance of Ca^{2+} sparks in the same single cell is almost always associated with the appearance of a STOC. (In the report by Nelson *et al.*, STOCs and Ca^{2+} sparks were recorded in different cells under different conditions.) Nelson *et al.* calculate that in smooth muscle cells, about 13 Ca^{2+}-activated K^+ channels are open at the peak of a STOC and suggest that this group of channels is activated by a single Ca^{2+} spark with an average peak value of 300 nM and an area of approximately 3 μm^2.

The Ca^{2+} sparks and the resulting STOCs appear to determine the degree of constriction of small arteries, and hence blood pressure. Agents such as ryanodine and thapsigarin, which interfere with Ca^{2+} sequestration by internal stores, cause membrane depolarization and arterial constriction. Iberiotoxin, which selectively blocks Ca^{2+}-activated K^+ channels, also causes membrane depolarization and vasoconstriction. And in a vessel treated with doses of ryanodine or thapsigarin, which prevent Ca^{2+} sparks and STOCs, iberiotoxin has no further effect. The implication is that under normal conditions, Ca^{2+} sparks that activate Ca^{2+}-sensitive K^+ channels in smooth muscle cells occur throughout the vessel wall. The local Ca^{2+} sparks and more widely spread hyperpolarizing currents exert a tonic hyperpolarizing and inhibitory influence that counters excitatory inputs to the smooth muscle cells.

We do not know what causes the Ca^{2+} sparks in these smooth muscle cells. Work on other systems has taught that opening of the ryanodine receptor channels is a bell-shaped function of $[Ca^{2+}]$ in the cytoplasm (*7*) and that increases in $[Ca^{2+}]$ inside internal Ca^{2+} stores enhance the probability of receptor opening (*8*). Because several agents that affect STOC frequency also affect various protein kinases (*1*), Ca^{2+} sparks may be triggered by local protein kinase activity. Various protein kinases affect ryanodine receptor function (*9*, *10*) and Ca^{2+} pumps on internal stores (*10*). It remains to be determined whether any of these factors triggers the Ca^{2+} sparks of smooth cells and, on the practical side, whether natural and synthetic vasodilators and vasoconstrictors act directly on the Ca^{2+} spark–STOC pathway.

The Ca^{2+}-activated K^+ channels function as endogenous Ca^{2+} detectors. Near the typical resting potential of smooth muscle (-40 to -50 mV), the value at which the experiments of Nelson *et al.* were carried out, a $[Ca^{2+}]$ of at least several micromolar is required for significant activation of these channels (*11*). The peak $[Ca^{2+}]$ detected during a Ca^{2+} spark was only about 300 nM. It is likely, therefore, that the Ca^{2+} sparks are much more localized but larger in amplitude than is revealed by these imaging methods. As new submembrane-localized Ca^{2+} indicators (*12*) and higher resolution imaging methods (*6*) are applied, these Ca^{2+} sparks will likely appear brighter and smaller. The average density of Ca^{2+}-activated K^+ channels on the smooth muscle cell membrane is estimated to be about 1 to 2 channels per μm^2 (*13*). Hence, to accommodate the coupling of a single Ca^{2+} spark—which causes the $[Ca^{2+}]$ to rise sufficiently to activate these K^+ channels in a region less than 1 μm^{2-} requires that these Ca^{2+}-activated K^+ channels are not uniformly distributed on the cell surface but instead are clustered in regions closely apposed to and in register with elements of the SR. Clustering of ion channels, exchangers, and pumps on the plasma membrane of smooth muscle has been recently observed (*14*) in close proximity to regions of the SR enriched in ryanodine receptors (*6*). Because Ca^{2+} is believed to diffuse very slowly through the cytoplasm (*15*), close approximation of Ca^{2+}-release sites to Ca^{2+}-sensitive targets is required, especially for the transmission of brief and small Ca^{2+}-release signals. Hence, the position of ryanodine receptors on internal stores relative to plasma membrane Ca^{2+}-activated K^+ channels and voltage-gated Ca^{2+} channels may well determine whether in a given cell ryanodine receptors amplify incoming Ca^{2+} currents or stimulate hyperpolarizing current as reported by Nelson and co-workers in cerebral arterial smooth muscle cells.

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