reinforces the view that a substantial proportion of DSBs are initiated by single energy absorption events. Earlier investigators demonstrated that induction of a DSB by a single •OH radical was a minor contributor to indirect damage, and they attributed the damaging effects to radical transfer between DNA strands (*9*). Boudaïffa and co-workers now propose a similar mechanism for low-energy electrons. They suggest that SSBs arise on each strand of the DNA after interaction with a single electron (*5*). Taken together, these findings indicate that transfer of radicals or energy between DNA strands may play an important part in amplifying the complexity of DNA lesions over and above the level set by the physical clustering of events along the radiation tracks.

Several mechanisms have been put forward to explain radical migration in DNA: swing-over of a sugar radical between the damaged and undamaged strands (*9*), attack by a base radical on a neighboring sugar (*7*), and radical reactions between adjacent bases (*10*). In general, the amplified damage follows the initial oxidative damage by •OH. In the mechanism proposed by Boudaïffa and colleagues—called "resonant attachment"—sub–ionization energy electrons attach to DNA, resulting in the formation of a transient molecular anion (see the figure). This is followed by either electron autodetachment (when no damage results) or bond dissociation. The latter results in either breakage of one strand or the modification of a DNA base, which leads to release of a radical fragment that can then migrate to and break the other strand.

The threshold energies for strand-break induction by photons and electrons are around 7 eV, well below the energy levels required for ionization (*2, 3, 5*). However, a comparison of the energy dependence of electrons with that of photons reveals an interesting difference: At an energy level of about 13 eV, electrons show a dip in strand-break efficiency. Below this energy level, resonant attachment is the dominant mechanism of DNA damage, whereas above it, nonresonant excitation is the primary cause of DNA strand breaks (*5*). The efficiencies of these low-energy interactions at inducing SSBs and DSBs per incident electron or photon are low—for example, 10^{-4} to 10^{-3} for 10-eV electrons (*5*) and 10^{-3} to 10^{-2} for 10-eV photons (*3*). However, this must be viewed in the context of the relatively high frequency of low-energy deposition events in a single radiation track (*1*).

There is currently much interest in understanding the effects of low-dose radiation on cells and molecules and how these effects relate to the risks for humans exposed to low-level radiation (*11*). Most existing knowledge of radiation risk comes from follow-up studies of atomic bomb survivors who received extremely high doses of radiation over very short time periods. Extrapolating these risk data to calculate risk for the very low doses that apply to typical environmental and occupational exposures requires the application of mathematical models (such as the linear-no-threshold model). But little is actually known about the biological effects of low-dose radiation. At the heart of the problem lies the need to unravel the actions of a single track of radiation on a cell (*12*). For example, at environmental levels of exposure, all cells in the body only "see" electron tracks at intervals averaging several months. A knowledge of how individual electron tracks interact with cells, their DNA, and other molecular constituents should lead to more refined models for calculating human risk at the exposure levels of most concern to the public and to regulatory agencies. Monochromatic beams of low-energy radiation are providing selective and specific ways to unravel the molecular mechanisms of damage induction. Intensive efforts to exploit the potential of low-energy electrons are under way at U.S., Canadian, European, and Japanese laboratories.

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PERSPECTIVES: SIGNAL TRANSDUCTION

The Calcium Entry Pas de Deux

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Calcium ions (Ca^{2+}) are universal secondary messengers that are key players in many cellular signal transduction pathways (*1*). There are two sources of these signaling cations in the cell: internal stores that release Ca^{2+}, and channels in the plasma membrane that open to allow external Ca^{2+} to flow into the cell. Internal Ca^{2+} stores—located in the sarcoplasmic reticulum of muscle cells and the endoplasmic reticulum (ER) of other cells—have a limited capacity for Ca^{2+} storage, and so they have to be replenished through entry of Ca^{2+} from the external environment. Putney (*2*) first recognized that the processes of emptying and replenishing internal Ca^{2+} stores must be linked. Somehow, empty Ca^{2+} stores activate store-operated channels (SOCs) in the plasma membrane that then allow Ca^{2+} ions to enter the cell. Putney termed this mechanism "capacitative calcium entry" (CCE) because the stores behave like a capacitor in an electrical circuit. When Ca^{2+} stores are replete the SOCs are closed, but once the stores discharge their contents, the SOCs open and Ca^{2+} ions enter the cell.

Since the first observations of CCE, there has been intense debate about the identity of SOCs and the way in which the ER Ca^{2+} stores communicate with them. Evidence is emerging in support of the popular conformational-coupling hypothesis (*3*, *4*), which proposes that information is transferred through a direct interaction between the inositol 1,4,5-trisphosphate receptor ($InsP_3R$) in the ER and SOCs in the plasma membrane (see the figure). On page 1647 of this issue, Ma *et al.* (*5*) now present evidence showing that CCE depends on the close proximity of the ER and plasma membranes and that $InsP_3Rs$ partner with SOCs to control Ca^{2+} entry through the plasma membrane.

The $InsP_3R$, embedded in the ER membrane, is a good candidate for this molecular coupling job. Its amino-terminal domain is large enough to span the 10-nm gap that separates the ER and the plasma membrane. Meanwhile, its carboxyl-terminal region forms a channel in the ER membrane, out through which flow Ca^{2+} ions in response to the signaling molecule inositol trisphosphate ($InsP_3$). It is this $InsP_3$-induced release of Ca^{2+} that normally depletes internal stores of the cation and results in activation of CCE.

Detection of light by photoreceptor cells in the compound eye of *Drosophila* activates a Ca^{2+}-entry channel known as TRP (transient receptor potential) in the photoreceptor cell membrane. Much excitement has surrounded the realization that mammalian cells express homologs of

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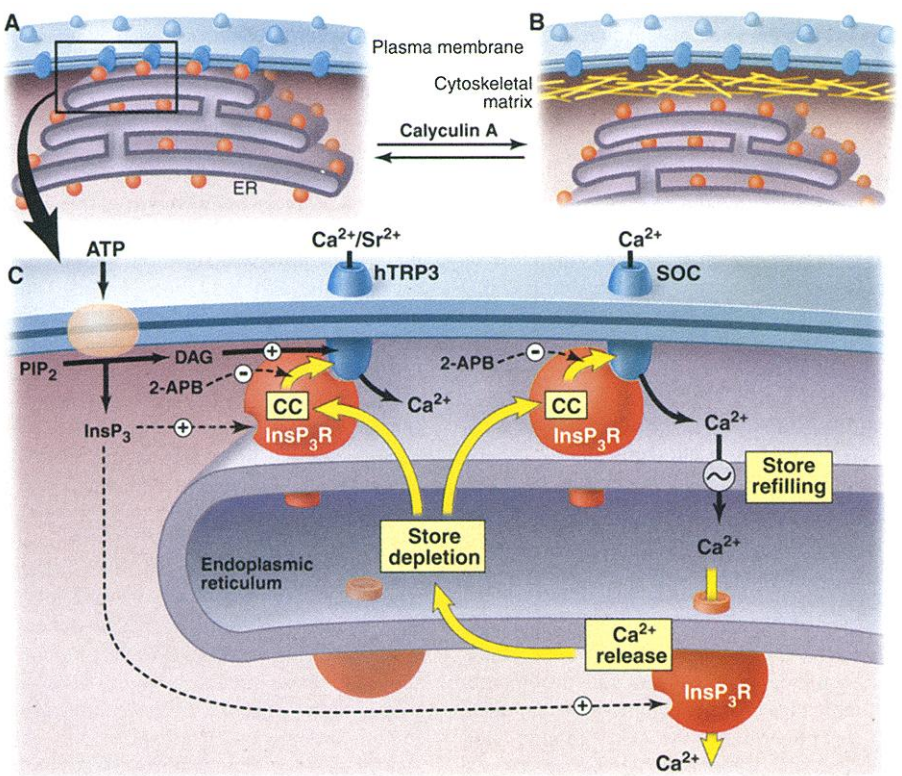
TRP and there has been speculation that one or more of these TRP homologs may be the elusive SOC. To date, seven TRP isoforms have been cloned that vary markedly in their channel conductance and mode of activation.

The human TRP3 channel (hTRP3) has been extensively characterized. It is clear that this channel is not activated solely by depletion of Ca^{2+} stores but rather also requires a direct physical connection to $InsP_3R$ (*5, 6*). Excised membrane patches containing hTRP3 channels can be activated by addition of vesicles containing the $InsP_3R$ bound to its ligand (*6*). Activation of hTRP3 can be blocked by rearranging the actin cytoskeleton; this displaces the ER membrane from sites of close apposition with the plasma membrane (*6, 7*) (see the figure). More significant still is the finding that hTRP3 and $InsP_3R$ form a signaling complex (they can be coimmunoprecipitated from membranes of human embryonic kidney cells overexpressing hTRP3) (*8*). Furthermore, high-affinity binding assays have been used to map the sites of interaction between the carboxyl-terminus of hTRP3 and the amino-terminus of $InsP_3R$ (*8*). Finally, engineering cells to express peptides that encode the interacting domains of either hTRP3 or $InsP_3R$ modulates the extent of Ca^{2+} entry (*8, 9*).

These studies clearly indicate that hTRP3 is activated by a conformational-coupling mechanism. But what about the identity of SOCs in mammalian cells? Perhaps the most exciting recent development has been the observation that experimental maneuvers that inhibit hTRP3 activity also prevent activation of endogenous SOCs. In their study, Ma *et al.* (*5*) show that displacement of actin in human embryonic kidney cells inhibits activation of both hTRP3 and SOCs (although, interestingly, the SOC response was not completely attenuated). In addition, both hTRP3 and SOC activity can be blocked with pharmacological agents—such as 2-aminoethoxydiphenyl borate (2-APB) and xestospongin C—that inhibit $InsP_3R$ activation (see the figure).

This new evidence thus implicates the $InsP_3R$ in the coupling of both hTRP3 and endogenous SOCs to Ca^{2+} entry after depletion of internal Ca^{2+} stores. The $InsP_3R$ is a central player in CCE by virtue of its ability to sense both cytosolic $InsP_3$ and empty Ca^{2+} stores. Although the mechanism of CCE is becoming clearer, we still do not know the identity of endogenous SOCs. In the strictest sense, TRP3 is not a SOC because it is not activated by Ca^{2+}-store depletion alone. The functional homology between TRP3, TRP6, and TRP7 suggests that they are activated in a similar manner, thus TRP6 and TRP7 are also unlikely to be SOCs. Furthermore, these TRP isoforms can be activated by diacylglycerol in an apparently store-independent manner (*5, 7, 10*). TRPs 1, 2, 4, and 5 are presently the best candidates for endogenous SOCs because, when expressed in cells, they are activated solely by Ca^{2+} store depletion.

Although cells may use subtly different conformational-coupling systems to activate various TRP isoforms, it seems that we are closing in on both the mechanisms and molecular players involved in CCE. But there are plenty of problems left to solve. For example, the expression pattern of mammalian TRPs is puzzling. TRPs are ubiquitously expressed yet are most abundant in excitable tissues, such as muscle and nerve, where CCE is least apparent. Another unresolved issue is the nature of the channels formed by TRPs when heterologously expressed (that is, when expressed in cells that normally have very low levels of the protein). The flow of Ca^{2+} ions through SOCs has been defined electrophysiologically as a Ca^{2+} release–activated current (I_{CRAC}) (*11*), which preferentially selects Ca^{2+} over monovalent ions. By contrast, cells expressing TRPs have Ca^{2+} channels that are not nearly as selective. Of all the isoforms, TRP4 comes the closest to resembling I_{CRAC}. On the positive side, however, antisense oligonucleotides directed against TRPs can prevent Ca^{2+} entry, supporting the notion that these proteins form endogenous CCE channels. It is plausible that the heterologous expression systems simply do not yet faithfully mimic the endogenous conformational-coupling complexes, and that other accessory proteins are required. Clearly, there is still much to learn about the enigmatic mechanism that determines how and when Ca^{2+} enters the cell.



A happy coupling. The conformational-coupling mechanism for the regulation of Ca^{2+} entry into cells. (**A**) The endoplasmic reticulum (ER) contains the inositol trisphosphate receptor ($InsP_3R$) (red), which interacts with store-operated channels (SOCs) in the plasma membrane (blue). (**B**) After addition of the phosphatase inhibitor calyculin A, a dense cytoskeletal matrix forms just below the plasma membrane, which displaces the ER and disrupts the molecular interaction between SOCs and $InsP_3Rs$. (**C**) Emptying of internal Ca^{2+} stores in the ER results in a conformational change (CC) in those $InsP_3Rs$ that are coupled to SOCs. In the case of endogenous SOCs (possibly TRPs 1, 2, 4, and 5), this CC is sufficient to activate opening of SOCs and Ca^{2+} entry from outside the cell. Store emptying, together with $InsP_3$ activation and diacylglycerol (DAG) production, may be necessary for activation of TRPs 3, 6, and 7.

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