monitoring the height fluctuations of lysozyme in the presence of a substrate (Radmacher et al., 1994). The Bustamante group has identified new morphologies for chromatin that have implications for the role of histone H1 (Zlatanaova et al., 1994), and L. Chang, F. S. Frank, P. Flicker, and D. Keller (personal communication) have used AFM to provide rather compelling evidence that both right and left handed forms of F-actin exist. Using a clever statistical analysis, Williams et al. (1994) have shown how aggregates can be analyzed to yield highly accurate particle size data. Thus, even rather poor images can be used to obtain valuable information on biologically important surfaces in realistic conditions.

The atomic force microscope is presently the instrument of choice for biological applications but, as discussed in a recent review I wrote for this journal (*Biophysical Journal* 67:937), the scanning tunneling microscope (STM) has a role in very high resolution imaging and in probing electronic effects. And, just to quell any impression that this is now a mature technology, the STM has surprises in store. The group of Guckenberger has provided dramatic illustrations of the way in which the STM, nominally limited to imaging good conductors, can, in fact, image on insulating surfaces in certain conditions (Guckenberger et al. (1994) and other material still in preprint form).

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Unraveling the Ryanodine Receptor

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Skeletal muscle activity is initiated by depolarization of the transverse (T) tubules, followed by release of calcium from the sarcoplasmic reticulum (SR). The ryanodine receptor (RyR), a 30S homotetramer, has been identified as the SR calcium release channel. The sequence of the single polypeptide predicts that 80% of the molecule at the N terminal side is hydrophilic and the remaining 20% forms the intramembrane channel. Thus, the unusually large size of the RyR (~2,200 kDa) is because of its cytoplasmic assembly, formed by the four subunits. The square shape of the cytoplasmic assemblies of RyRs have allowed their identification with the feet, structures located in the gap between SR and T tubules. In skeletal muscle, the voltage sensors of e-c coupling, the dihydropyridine receptors (DHPRs), are probably linked to the RyRs, so that a molecular interaction between the two is possible. Another calcium release channel of the endoplasmic reticulum (the IP3 receptor) has sequence homology to the RyR, and an analogous structure, but it does not interact with the surface membrane. Two recent papers have added important new information on the ryanodine receptor by providing a high resolution image of the channel, and identfying a ligand binding site.

Understanding of the control of RyR permeability during activation of muscle contraction and of The RyR's response to various ligands requires knowledge of the protein configuration. A paper in press in the *Journal of Cell Biology* (Radermacher et al., 1994) and a report in this issue of *Biophysical Journal* by Wagenknecht et al. give a 3-D reconstruction of the channel at ~3 nm resolution, and the location of the calmodulin binding site. The purified, detergent solubilized channel was imaged in the EM. Three powerful approaches were used: cryoEM, which allows structural preservation to high levels of resolution without stains or fixatives, image averaging, and conical tilt reconstruction, which permits three dimensional reconstruction from a random field of molecules, using only a pair of images. In both papers, correspondence analysis allowed classification of the images into classes and averaging of information from a number of structurally homogeneous molecules selected in an unbiased fashion. The data provide a framework, which will be the basis for an atomic model of the RyR.

The reconstructed RyR (Radermacher et al., 1994) shows a clear demarcation between the channel and cytoplasmic assemblies, both with fourfold symmetry. The cytoplasmic assembly is tall, thus providing a physical link between SR and T tubules. Several details of its structure are noteworthy. It is highly hydrated, with the architecture of a scaffolding designed to provide a mechanical linkage between the two membranes while allowing excellent opportunity for flow of calcium from the channel to the myofibrils. In addition, a possible direct pathway for calcium exit is seen near the junction of the transmembrane and cytoplasmic assemblies. The four corners of the molecule have a complex outline with grooves that seem appropriate for interdigitating with neighboring molecules, as seen when the molecule forms arrays in situ. The three highest domains of the cytoplasmic assembly, near its corners, are likely to be those interacting with the DHPRs. Finally, the most important consideration is the link between the cytoplasmic domain, which presumably has binding sites for regulators of the channel properties (e.g., DHPRs and calmodulin, see below) and the channel that is being regulated. Only one connection is seen between the cytoplasmic and transmembrane domains, making it a somewhat tenuous link. However, a central plug-like mass in the channel is sufficiently close to this connecting domain that an interaction is possible. As usual, details of the intramembrane region of the molecule

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are not as well resolved as those of the cytoplasmic region, and the question of the number of membrane helices (4 vs. 12) is still open. A low density channel through the central region of the molecule might be the permeating pore; however, the question of single versus multiple conductance pathways is certainly not solved for this channel.

We now have a fairly detailed general plan of the RyR, and the next step is to identify the functional sites. Wagenknecht et al. (1994) map the calmodulin binding site. Calmodulin, in the nanomolar range, directly inhibits calcium release through the RyR, and potential calmodulin binding sites have been identified in the hydrophilic region of the sequence. Taking advantage of a cystein residue (Cys-27), calmodulin was tagged with a monomaleimido derivatized 67-atom gold cluster, which can be directly detected with a precision of about 2 nm (a relatively small distance in a molecule which is 29x29x12 nm). A second approach, using biotinylated calmodulin and its detection by streptavidin-gold, has lower resolution, but better visibility of the probe. Averaged images with the smaller complex show the gold label in symmetrically located regions near the corners of the molecule. Side views with the larger probe show the binding on the side of the protein facing T tubules, which is at some distance from the channel assembly.

It is interesting that the binding sites for DHPR and calmodulin predicted by this work are near the corners of the cytoplasmic assembly, far away from the channel. One is reminded of the structure of the SR calcium pump, in which the nucleotide binding and the phosphorylation domains are at relatively large distance from the high affinity calcium binding site. In the case of the ryanodine receptors, chain interactions responsible for the single high affinity binding site for ryanodine (Carrol et al., 1991) and for the shift from single to multiple conductance states (Liu et al., 1989) will have to be defined. FK506-binding protein (a proline isomerase of 12 kDa associated with the RyR, Jayaraman et al., 1992) stabilizes the channels into the full conductance level (Brillantes et al., 1994), while in its absence the channel exhibits four distinct conductance states.

The process of establishing structure-function correlations in the RyR, leading to the understanding of e-c coupling, is well on its way. A large number of ligands are known to affect the channel (reviewed by Coronado et al., 1994). Positions of ligand binding sites in the primary structure are being predicted from the primary sequence, and determined by the use of proteolysis and of fusion proteins coupled with sequence specific antibodies. The high affinity ryanodine binding site, for example, seems to be buried somewhere close to the channel region of the molecule (Callaway et al., 1994). The two papers discussed here provide strategies for visualization of the tertiary structure of RyRs and the location of ligand binding sites within it. We will be eagerly waiting for the next installment, including a direct visualization of conformational changes accompanying opening of the channel.

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