coupling in skeletal muscle. The apparent identity of the kinetics of activation of the skeletal and cardiac ryanodine receptor Ca^{2+} channels to photolyzed Ca^{2+} indicates that the difference in excitation-contraction coupling mechanisms in these two types of muscle are unlikely caused solely by differences in ryanodine receptor subtypes.

To reconcile their data with the existing literature on excitation-contraction coupling in skeletal muscle Györke et al. (1994) suggest that the Ca^{2+} activation site of the skeletal muscle ryanodine receptor may be morphologically restricted in situ, or that physiologically important ligands may be omitted in the planar lipid bilayer experiments. Such ligands may either stimulate or inhibit the ryanodine receptor Ca^{2+} channel and they may be specific for the skeletal or cardiac isoform of this channel protein. In support of this latter proposal, Fruen et al. (1994) have recently shown that a number of inorganic anions activate the skeletal, but not the cardiac, muscle ryanodine receptor Ca^{2+} channel. Thus, other yet undiscovered ligands that specifically regulate the skeletal muscle ryanodine receptor Ca^{2+} release channel may ensure that the activation of these two ryanodine receptor isoforms are very different in situ. What other treats are in store for us using this novel adaptation of the flash photolysis procedure are awaited with great interest by those studying excitation-contraction coupling in muscle.

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Toward Unified and Consistent Views of Protein Dynamics

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The physical and functional consequences of internal protein dynamics have long been of interest (see Frauenfelder et al. (1990) and references therein). In many respects, however, the various time scales and corresponding manifolds of dynamics have been artificially isolated by the techniques used to probe and understand them. Thus, for example, there has been little reconciliation historically between the long time scale dynamical behavior seen in computer simulations with comprehensive experimental observations. Indeed, this pattern of isolated investigation is seen even among experimentalists concerned with biopolymer dynamics on the same time scale. A classic example might be the combined application of fluorescence and NMR techniques to characterize the subnanosecond motions of proteins. In a paper in this issue of *Biophysical Journal* that, one would hope, will initiate a trend in these kinds of studies, Kemple and co-workers use the dynamical information inherent in both fluorescence and NMR spectroscopy to characterize tryptophan side-chain motions in the native and two single tryptophan mutant forms of *E. coli* thioredoxin.

This paper is timely in many significant respects. The methodologies of both NMR and fluorescence spectroscopy have recently emerged as mature, sophisticated, and powerful technologies. This is especially true for NMR, where tremendous strides have been made in the methodologies employed in the solution of the resonance assignment problem (Bax and Grzesiek, 1993), the determination of the solution structures of proteins (Clore and Gronenborn, 1991) and, most recently, in the characterization of the internal dynamics of proteins by use of NMR relaxation phenomena.

After a rough start where concern was raised about the experimental and analytical strategies of early approaches (Boyd et al., 1990; Peng et al., 1991; Dellwo and Wand, 1991), the application of NMR relaxation techniques to the exploration of the fast internal dynamics of proteins has resulted in the study of two dozen or more systems in the past few years. Most have involved characterization of main-chain dynamics by use of ^{15}N relaxation. Kemple and co-workers move the dynamic issue to the more interesting realm of internally buried side chains and employ ^{13}C relaxation phenomena. The combined use of both NMR and fluorescence phenomena allow Kemple and co-workers to explore with some confidence a number of issues that have plagued isolated studies. These have to do with origins of artifacts and inaccuracies such as complete accommodation of chemical shift anisotropy, the functional form of the spectral density, and the nature of global tumbling, which must enter into consideration explicitly. The functional form of the spectral density arising from the so-called model-free treatment of Lipari and Szabo is especially appealing on several grounds. This treatment provides for relatively accurate descriptions of the internal motions underlying relaxation in terms of two root parameters: the so-called generalized order parameter and the effective correlation time. The former provides a measure of the spatial amplitude of the motion, and the latter effectively provides an upper limit on the correlation time(s) of the motion(s) contributing to relaxation. There is no requirement to specify the exact physical nature of the internal motion beyond the requirment that they

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be Markovian or diffusive-like (see below). Although perhaps misnamed (it might be better called the model-insensitive approach), the model-free treatment is also particularly attractive because it has been employed in both NMR and fluorescence spectroscopy and thereby explicitly emphasizes the fundamental similarity of the information available from the two techniques. And, most important, it emphasizes the requirement that consistent views of the motions observed be obtained.

Kemple and co-workers pay particular attention to these issues and provide a careful summary of the underlying physics and analytical strategies employed by these two approaches. Origins of discrepancies are meticulously examined. Although in the end the novel and new results presented by this paper are limited, it does, as a whole, point to the direction investigators might go. Questions to be probed include those implicitly posed by Wagner: Can the motions occuring in proteins on the subnanosecond time scale be described by simple Lorenztian spectral densities?; Equivalently, in the language of Frauenfelder et al. (1990), are these motions occuring in simple and homogenous energy wells or is the "energy landscape" over which these motions travel much more complex? Ultimately, the kind of data obtained by the use of NMR and fluorescence relaxation will provide the strongest possible test of the accuracy and validity of the details of molecular dynamics calculations. This is because both spectroscopic techniques are dominated by "equilibrium fluctuations" that should be consistently manifested in molecular dynamics trajectories of sufficient length. All of these questions and issues have great significance for our understanding of protein structure, dynamics, stability and, ultimately, function. These are also questions that NMR spectroscopy can properly assist in answering, but only if there is significant confidence, flexibility, and robustness in the general approach. Such confidence is engendered by careful and comprehensive studies such as those presented by Kemple et al. (1994).

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