The conformational propensity of amino acids in the unfolded state of peptides and proteins is the subject of ongoing deliberation. Recent research has mostly focused on alanine, owing to its abundance in proteins and its relevance for the understanding of helix coil transitions. We have analyzed the amide I' band profiles of the IR, isotropic and anisotropic Raman, and VCD profiles of a series of GXG peptides, X representing a subset of the naturally occurring amino acids, in terms of a conformational model which explicitly considers the entire ensemble of possible conformations rather than representative structures. The distribution function utilized for satisfactory simulations of the amide I' band profiles was found to also reproduce a set of seven J-coupling constants reported by Graf et al[1]. The results of our analysis reveal a PPII fraction of 0.91 for the central alanine residue in GAG, which strongly corroborates the notion that alanine has a very high PPII propensity. We performed a similar analysis for X=E, F, S, V, K, L and M. Preliminary indication is that E, F, K and L exhibit a substantial PPII propensity, whereas S, V and M exhibit a less pronounced PPII propensity with an increased propensity for β-strand. We also used distributions from coil libraries and MD simulations to model amide I band profiles and J-coupling constants for alanine and valine. We found most of them to be inconsistent with our experimental data. Thus, these results clearly demonstrate that caution must be taken in using coil libraries and MD simulations to describe the unfolded state of peptides and proteins, and that experimental data are a prerequisite for quantitative evaluation of amino acid residues.

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Intrinsic Disorder and the Evolution of Viral Overlapping Genes

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Overlapping genes result from frameshifts over pre-existing genetic sequences by a process called overprinting, yielding two or more different proteins encoded by the same nucleotides. In this situation, single base changes would frequently alter the amino acids in two or more proteins simultaneously, but such mutations would need to fit two or more sets of structural constraints. One way around such double or multiple constraints would be for overlapping genes to be enriched in structural disorder, which is more tolerant of mutations than is structure. To test this idea, we predicted structure/disorder in the protein products of manually curated overlapping genes from unspliced RNA viruses. Overlapping regions were found to be significantly more disordered than non-overlapping regions and to have a sequence composition biased towards disorder-promoting amino acids. For a subset of the overlapping genes in our dataset, the genetic sequences that were created *de novo* by overprinting of ancestral genes were determined. Most of the protein products of these novel genes are disordered and have unusual amino acid compositions. Furthermore, almost all of these gene products are accessory proteins rather than replicases or other proteins fundamental to viral replication or structure, and these proteins are orphans without homologues. Proteins that have been created by overprinting different homologues of the same genes display a diversity of functional and structural features, facts that are consistent with their *de novo* origin. Our results offer a glimpse of the structural and functional characteristics of protein regions encoded by genes created *de novo* by overprinting events in viruses. In most cases, intrinsically disordered gene products seem to help alleviate both the difficulty of generating structured proteins *de novo*, and the increased evolutionary constraints expected for multiple-coding genetic sequences.

Platform Y: Voltage-gated Ca Channels

1141-Plat

Persistent Increases In Ca^{2+} Influx Through Cav1.2 (I_{CaL})Induce Cardiac Conduction Disturbances And Sarcoplasmic Reticulum Ca^{2+} Overload To Induce Cardiac Arrhythmia And Sudden Death

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Increases in Ca^{2+} influx through Cav1.2 has been observed in cardiovascular disease (CVD) and associated with cardiac arrhythmias. **Methods**: To mimic the enhanced Cav1.2 activity in CVD, we overexpressed the Cav1.2 β2a subunit in a transgenic mouse model. In-vivo ECGs, ion currents and intracellular Ca^{2+} were measured in transgenic (TG) and control (CTR) mice. **Results**: I_{CaL} was greater in TG myocytes (23.9 ± 2.5pA/pF, CTR 13.8 ± 1.6pA/pF). TG mice had enhanced cardiac performance (EF: TG 72.7 ± 1.3%, CTR 66.7 ± 1.5%) but died suddenly (TG 50% vs CTR 100% alive at 6 months), suggesting cardiac arrhythmias. In conscious mice, there was no difference in heart rate in CTR (571 ± 29bpm) and TG (541 ± 24bpm) mice but the QT interval was significantly shorter in TG (44.0 ± 5.5ms, CTR 58.2 ± 3.4ms) mice. Second degree AV block and ectopic premature ventricular beats were observed in all 4 TG mice but not in CTR mice. In anesthetized mice, there was no difference in heart rate (CTR 513 ± 20bpm, TG 526 ± 13bpm) but the PR interval (CTR 32.4 ± 1.4ms, TG 49.8 ± 6.2ms) and QRS duration (CTR 11.4 ± 0.8ms, TG 14.5 ± 0.8ms) were significantly prolonged in TG mice, indicating conduction defects. A significantly greater % of TG myocytes (28.5%) had early (EADs) and delayed (DADs) afterdepolarizations than CTR (0.0%) due to enhanced SR load (caffeine spritz and Fluo-4 F/F_0: TG 4.7 ± 0.4 vs. CTR 3.2 ± 0.3) and I_{NCX} (TG 2.15 ± 0.6pA/pF vs CTR 1.12 ± 0.3pA/pF at +60mV). However, action potential duration (APD) was significantly shorter in TG myocytes (APD90%: 40.0 ± 5.7ms vs. CTR: 100.6 ± 15.2ms) resulting from an increase of Ito (TG vs. CTR: 60.2 ± 0.8pA/pF vs. 18.7 ± 3.0pA/pF at +50mV). **Conclusion**: Persistent increases in Ca^{2+} influx through Cav1.2 cause both conduction disturbances and SR Ca overload, and induce cardiac arrhythmias with shortened APDs and QT intervals.

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Rem Selectively Abolishes β1-adrenergic Regulation Of $Ca_V1.2$ Channels In Heart

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β-adrenergic modulation of cardiac $Ca_V1.2$ channels is critical for sympathetic regulation of the heartbeat, and its disruption is a harmful hallmark of heart failure. RGK (Rem, Rem2, Rad, Gem/Kir) GTPases potently inhibit Ca_V channels by interacting with their auxiliary β subunits. Intriguingly, RGK proteins are present in heart, and their levels are elevated in heart failure. We examined the impact of the RGK GTPase, Rem, on $Ca_V1.2$ channels in heart cells and assessed whether there was crosstalk with the β-adrenergic modulation of the channel. Cultured adult guinea pig ventricular myocytes expressed robust $Ca_V1.2$ currents ($I_{Ca,L}$) (15.08 pA/pF) and responded to β1-adrenergic activation (1 μM isoproterenol + 1 μM ICI118,551) with a sharp, three-fold increase in current density. Isochronal cardiac cells expressing YFP-Rem, achieved through adenovirus infection, displayed a markedly lower basal current density (5.85 pA/pF). Nevertheless, the effect of Rem in heart is quantitatively smaller than seen in recombinant channels expressed in HEK 293 cells, which feature a virtual ablation of $I_{Ca,L}$. Surprisingly, the remaining Rem-insensitive $I_{Ca,L}$ in guinea pig heart cells was essentially unresponsive to β1-adrenergic stimulation. This was not due to disruption of the signaling pathway because isoproterenol-mediated increase in cardiac I_{Ks} remained unchanged. Intriguingly, the Rem insensitive $I_{Ca,L}$ remained responsive to forskolin. These results reveal an unexpected crosstalk between RGK GTPases and β-adrenergic signaling pathway at the level of cardiac $I_{Ca,L}$, and suggests that Rem selectively inhibits spatially distinct $Ca_V1.2$ channels in single heart cells.

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The Timothy Syndrome Mutation In Cav1.2 Causes Dendritic Retraction Through Calcium-independent Activation Of The Rhoa Pathway

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L-type voltage-gated calcium channels (LTCs) play a key role in neuronal development by activating signaling pathways that regulate neuronal gene expression and morphology. A point mutation in the LTC CaV1.2, which blocks voltage-dependent inactivation (VDI), causes autism in Timothy Syndrome (TS) patients. While it is known how VDI influences the current through LTCs, it is not known how alterations in VDI affect the signalling function of CaV1.2 in neurons and ultimately cause developmental defects that lead to autism. Here we show that CaV1.2 channels containing the TS mutation (TS-CaV1.2) cause dendrite retraction and reduced dendrite branching in cortical neurons in vitro and in vivo. Surprisingly, we found that TS-CaV1.2 causes dendritic retraction independently of Ca2+ influx through the channel suggesting that the voltage-dependent conformational changes associated with VDI play an important and unexpected role in CaV1.2 signaling. In addition, we found that TS-CaV1.2 causes dendrite retraction by activating the RhoA signalling pathway. We found that the small GTP-binding protein Gem with the channel beta subunit play a critical role in mediating the calcium-independent activation of RhoA by TS-CaV1.2. Our results provide new insight