and UAA (ochre). These stop codons are occasionally recoded to an amino acid by specialized transfer RNAs (tRNAs) called suppressor tRNAs. In all organisms, the UGA codon is recoded to selenocysteine (Sec; the 21st amino acid), depending on the downstream enhancer sequence SECIS, through multi-step Sec synthesis on tRNA-Sec (opal suppressor). In methanogenic archaea and some anaerobic eubacteria, the UAG codon is recoded to pyrrolysine, the 22nd amino acid, through direct acylation of amber suppressor tRNAPyl by pyrrolysyl-tRNA synthetase (PylS). The tRNAPyl suppressor has an unusual structure, lacking most of the consensus features of canonical tRNAs, analogous to mammalian mitochondrial tRNAs. It is thus unclear how such unusual tRNAs function in the ribosome and how PylS discriminates between suppressor and canonical tRNAs. Here we present the crystal structure of the PylS/tRNAPyl complex from Desulfitobacterium hafniense at 3.1 Å resolution. The suppressor tRNAPyl has an unusual minimal core structure formed by a non-standard D-loop, a TYC-loop and a variable loop, which are re-organized to mimic the canonical L-shape to function normally in the ribosome. PylS recognizes the unusual compact core structure by steric compatibility, which enables discrimination of the suppressor from the other canonical tRNAs.

Keywords: tRNA, aminoacyl-tRNA synthetase, stop codon

**MS.08.4**

*RNA-protein interactions in the U4 snRNP core domain*

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The spliceosome responsible for processing pre-mRNA to mRNA is assembled from RNA-protein particles called snRNPs. The U1, U2, U4 and U5 snRNPs contain in their core a common set of seven Sm proteins (B/B′, D1, D2, D3, E, F and G), which assemble on the particle-specific snRNA (small nuclear RNA) by collectively recognizing a conserved U-rich heptad sequence called the Sm site. Addition of particle-specific proteins to the core assembly completes the mature snRNP. The Sm proteins share a conserved sequence motif in two segments, called Sm1 and Sm2, joined by a linker of variable length. We have reconstituted and crystalized the human U4 snRNP core domain comprising the U4 snRNA Sm site with flankingstem-loops and the seven Sm proteins. The crystals belong to space group P31 (α = 248.0 Å, c = 251.9 Å) with 12 copies of the core domain in the asymmetric unit. They diffract X-rays to 3.6 Å resolution. The structure was solved by a series of MAD experiments using SeMet-substitution of different groups of Sm proteins, followed by molecular replacement to the native crystal. Our results show that the seven Sm proteins form a closed ring through H-bonding across the Sm1, Sm2 motifs all the way round, and the snRNA is threaded through the pore. The ring has a flat side where the N-terminal helices lie, and a conical side where the linkers protrude. At the rim where the 5′RNA enters the pore from the flat side, the seven Sm proteins bind the Sm site heptad (AUUUUUG) in a one-to-one but asymmetric manner. In addition, the proteins make non-sequence-specific interactions with the 5′ and 3′ stems. Our findings are applicable to other snRNP cores, because of the conservation of the Sm site and constancy of the Sm proteins.

Keywords: RNA splicing, crystal engineering, noncrystallographic symmetry

**MS.08.5**

*Structural basis of lipid biosynthesis regulation in Gram-positive bacteria*

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FapR is a global transcriptional repressor that controls the expression of several genes involved in the biosynthesis of fatty acids and phospholipids in many Gram-positive bacteria. The FapR monomer has a helix-turn-helix motif linked to an effector-binding domain that displays a ‘hot-dog’ fold, similar to that of several thiostrepton known to process acyl-CoA substrates but different from other known bacterial transcriptional regulators (Schujman et al, 2006). Binding of the specific inducer, malonyl-CoA, promotes conformational changes in the protein that causes the FapR-DNA complex to dissociate or prevents its formation. Furthermore, site-directed mutations which disrupt the FapR-malonyl-CoA interaction result in a lethal phenotype in Bacillus subtilis, suggesting that this homeostatic signaling pathway could be a target for novel chemotherapeutic

**MS.08.3**

*DNA transfer machines*

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Whatever the route used, horizontal gene transfer, a rapid way for bacterial evolution, requires sophisticated protein machinery to enable DNA to cross the cell envelope barriers. Increased antibiotic resistance among pathogens is a troubling consequence of this microbial capacity. Mechanisms leading to horizontal gene transfer in bacteria are categorized into transduction, transformation and conjugation. Transduction occurs via bacteriophages, which may incorporate portions of the host bacterial DNA and introduce them into newly infected hosts. Natural transformation consists of the uptake of naked DNA from the environment. Finally, conjugation is the unidirectional transfer of ssDNA of conjugative plasmids from a donor to a recipient cell. The conjugation system can be divided into two discrete specialized modules: the relaxosome, which triggers plasmid DNA processing and replication, and a type IV secretion system, which impels protein and single-stranded DNA through the membranes. In addition, a coupling protein, which links these two modules, and a number of ancillary proteins are required. Over recent decades, research efforts in the field have clarified many aspects of the system. In particular, structural biology is providing details of the molecular architecture of several of the components involved. Russi, S., Boer, R. & Coll, M. (2008). Molecular Machinery for DNA Translocation in Bacterial Conjugation. In Plasmids: Current Research and Future Trends. Horizon Scientific Press, Londres.

Keywords: protein-DNA complexes, DNA translocation, bacterial conjugation