RIBOSOMAL BASIS OF HAVOC IN MOLECULAR HYPOMETABOLISM:

NEW INSIGHTS INTO ACTINOBACTERIAL EFFECTORS

NEW THOUGHTS FOR THE FUTURE

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OR, DO WE DARE SPECULATE ON SMALL PROTEIN EFFECTS ON PD AND P.ACNES?

- METABOLOMICS OF SEBUM REVEALS LIPID DYSREGULATION IN PD
- ALTERATIONS IN CARNITINE SHUTTLE, SPHINGOLIPIDS, ARACHIDONIC ACID AND FATTY ACID METABOLISM
- CORRELATED WITH PD AND SEBUM
- SEBUM TO P. ACNES TO RETE RIDGE TO DOPAMINERGIC TISSUES?

CONFLICT OF INTEREST

- ROYALTIES FROM SALES ON:
  - SURVIVING MOLD
  - PROGENEdx

GOALS FOR TODAY

- A SENSE OF WONDER FOR SMALL MOLECULE RIBOSOMAL INHIBITORS, NUMBER, DIVERSITY, COMPLEXITY
- A SENSE OF WONDER AT JUST HOW FAR THIS TOPIC GOES
- STRUCTURE OF AN ACTINOBACTERIA STUDY
- APPLICATIONS TO IMMUNOLOGIC BUILDING CLEANING: PARKINSON'S

IN CASE I FORGET, P. ACNES

- PERSIST IN EPITHELIAL CELLS/CRYPTS
- MACROPHAGES TOO, TO CAUSE SARCOID
- MOST TANTALIZING, INFILTRATING BRAIN PARENCHYMA TO CONTRIBUTE TO NEURODEGENERATIVE CHANGES, ESPECIALLY PARKINSON'S SYNDROME, PERHAPS VIA GLYCOLIC ACID-BASED VESICLES
- P. ACNES TRAVERSES BBB VIA TRANSCELLULAR INVASION AIDED BY LPS, E-SELECTIN, ICAM-1, VCAM-1

RIBOTOXINS UBIQUITOUS IN ARCHAEA

- FUNGI
- BACTERIA
- ACTINOBACTERIA
- FAB!!
- CLEANED BUILDINGS WON'T HAVE FAB??
- NO WAY!

CIRS AROSE FROM INFLAMMATION FOLLOWING BIOTOXIN EXPOSURE

- BIOTOXIN RELEASE ELICITED BY GENE SIGNALING
- TODAY'S FOCUS, AND ITS ENORMOUS POTENTIAL, STEMS FROM EXTRACELLULAR RELEASE OF RIBOTOXINS
  - RELATED TO LONG INCUBATION PERIOD???
- A NEW MECHANISM OF CELLULAR INJURY?
- NO! AN EVOLUTIONARILY CONSERVED OLD MECHANISM

GETTING STARTED, SIMPLY

- Ribosome targeting initiation inhibitors.
- Ribosome inhibitors affecting termination.
- Ribosome recycling inhibitors
- Inhibitors of eukaryotic translation factors
- Elongation too

LOTS MORE

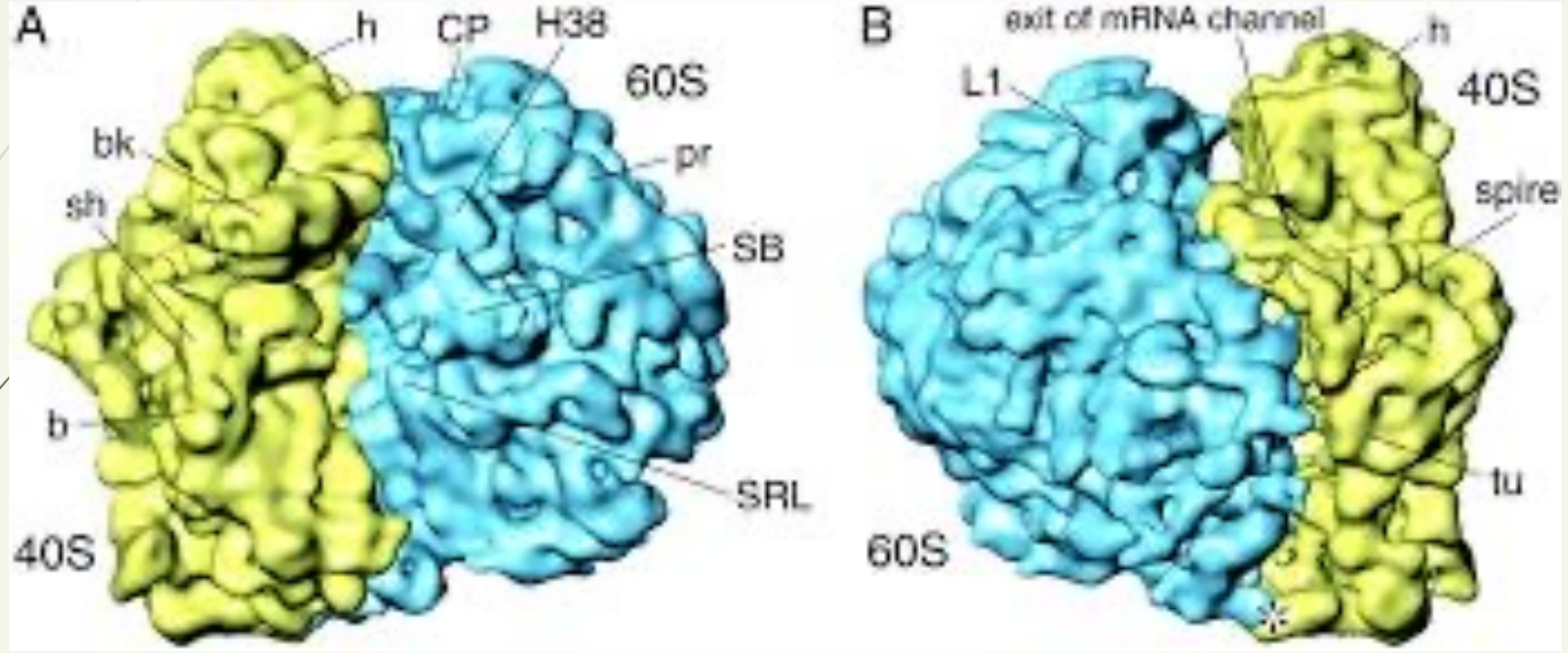
- Inhibitors blocking the polypeptide exit tunnel.
- Translocation inhibitors
- Drug inducing decoding errors especially paromomycin.
- Other mechanisms of elongation failure.

Sarcin/ricin loops get a lot of attention, but mechanisms of injury are diverse

- The array of small molecule drugs known to interact adversely with translation are a broad array with specific inhibition of protein synthesis in eukaryotic cells.
- Some block translation
- Others block translocation
- Peptidyl transferase center and polypeptide exit tunnel are targets
- Others will modulate the binding of translation machinery components to the ribosome
- Others induce miscoding, premature termination or stop code.

MANY OTHERS

- Other compounds inhibit aminoacyl-tRNA synthesis, translation factors and components of translation pathways including MTOR.
  - Especially in Parkinson's IF MTOR signaling blocks cell death
- The diversity of properties that these compounds have is phenomenal: including antidepressant or immunosuppressive
- Translation inhibitors are a hot item research for inhibiting eukaryotic protein synthesis classified by their targets.
- In this seminal paper, the authors present initially 370 inhibitors of eukaryotic protein synthesis; then have a supplement with 50 more.
- **Thanks to Gianni Rossini**
- **Dmitriev S, Vladimirov O, Lashkevich K. A quick guide to small-molecule inhibitors of eukaryotic protein synthesis. Biochemistry 2020; 85: 1389-1421.**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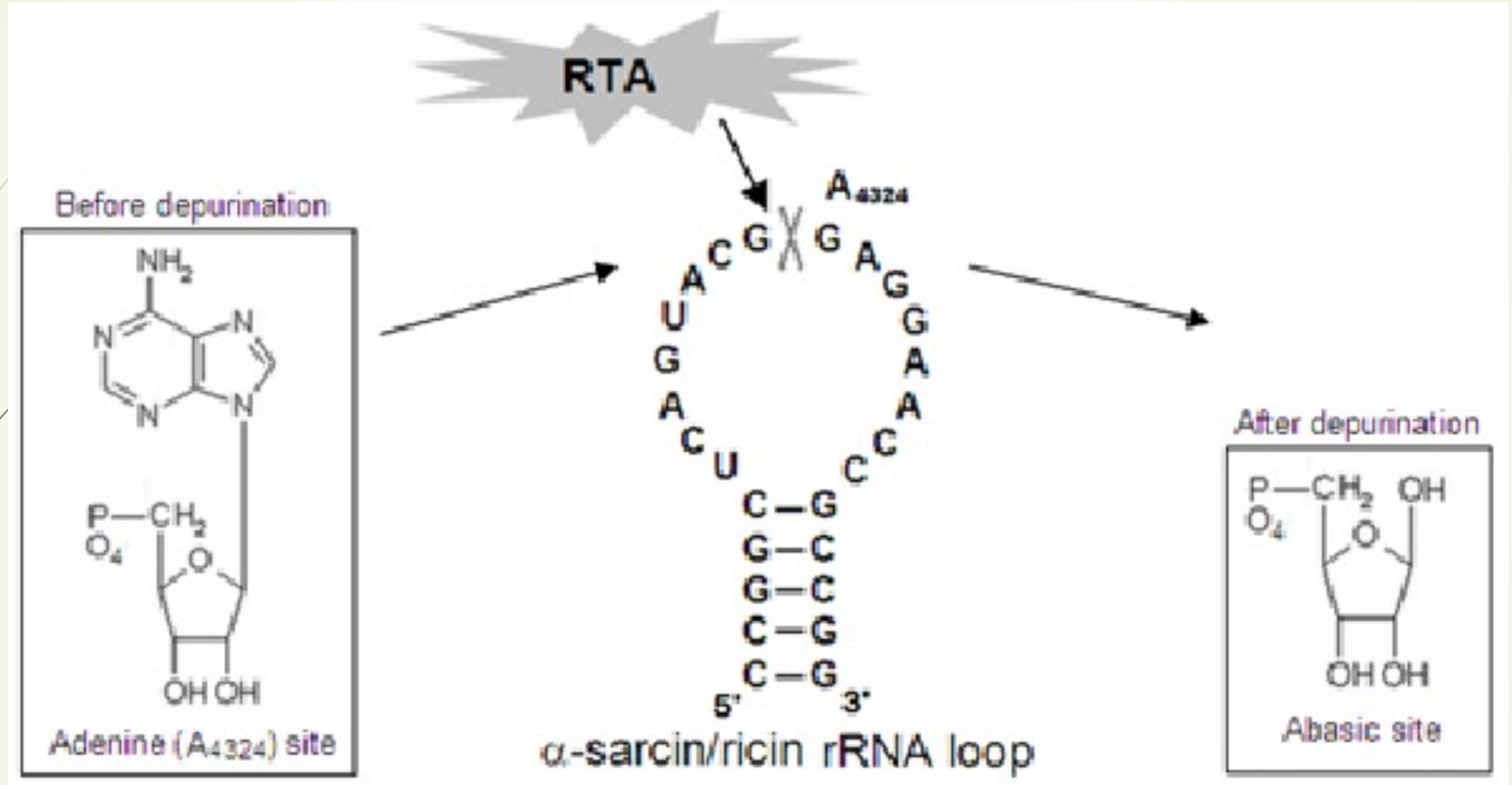
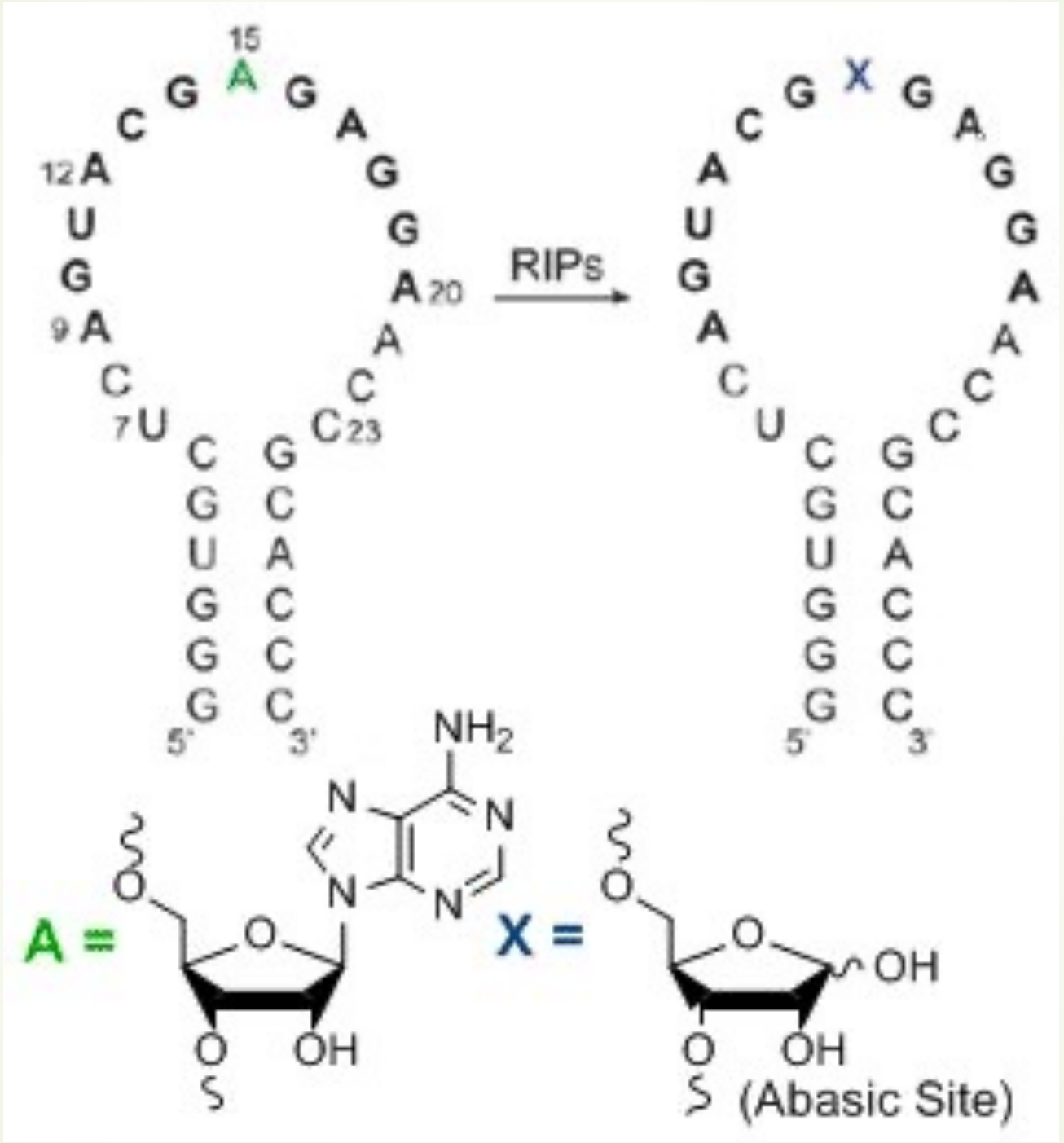

RTA
Before depurination
Adenine (A4324) site
A4324
α-sarcin/ricin rRNA loop
5'
3'
After depurination
Abasic site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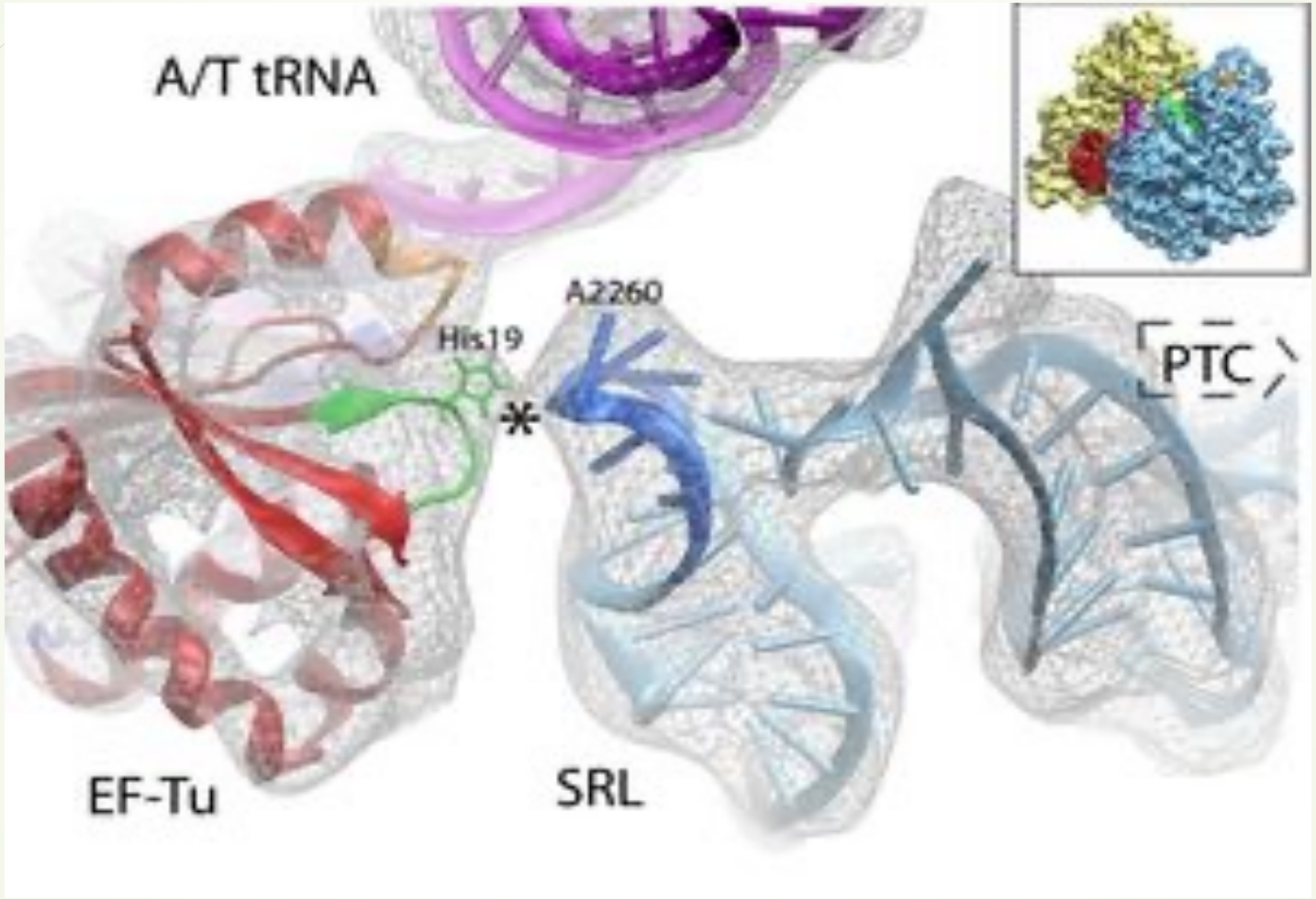
A/T tRNA
A2260
His19
PTC
EF-Tu
SRL

This paper will note the sarcin ricin loop inhibitors but the scope will include the diverse functions of ribosomal inhibitor proteins (RIPs) and ribotoxins. These compounds create the ribosomal stress response

- Ribosomal active compounds can be further defined by the source. Trichothecenes are well known inhibitors of ribosomal function made by fungi. Similarly, Actinobacteria, well known for the ability to make thousands of compounds, are well represented by their ability to attack ribosomal function.
- Puromycin and lactimidomycin, actino inhibitors of ribosomal function, are well defined in the literature. Puromycin, a premature termination inducer, can exhibit concentration dependent effects or trigger ribotoxin other types of stress living cells.

INHIBITORS OF EUKARYOTIC RIBOSOMES

- Ribosome-targeting elongation inhibitors are the overwhelming majority of known ribosomal targeting compounds. These include inhibitors of peptidyl transferring reaction and translocation; peptidyl tunnel blockers; and inducers and miscoding of premature termination.
- The peptidyl transferring center structure (PTC) is conserved over time making it a vulnerable spot “protein synthesizing machinery.” In both bacteria and eukaryotes, the largest number of inhibitors bind at the site. That is not to say that only one compound will bind to the PTC; it is populated with great diversity.

Greater complexity

- Another specific actinobacterial inhibitor, anisomycin, interacts with a site on the aminoacyl-tRNA entry area and destabilizes the tRNA binding. This is the same site that is targeted by trichothecenes (T-2 toxin) and deoxynivalenol, and at least 3 dozen similar compounds, all having a four-member heterocyclic ring created by a fungus. The four-member heterocyclic rings are more commonly found in plant bacteria.
- Notably, plants make different PTC binders, including harringtonine.
- This drug has been modified to become a useful treatment for chronic myeloid leukemia.

Greater complexity yet

- Another actinobacteria ribotoxin, sparsomycin, attacks the PTC.
- It is been shown to interact with the large ribosomal subunit of Archaea; This compound can make multiple contacts with tRNA and while simultaneously preventing the binding of the aminoacyl-tRNA to a specific site.
- Here we meet the difficulty classifying PTC inhibitors based on the stage blocked.
- The situation is more complicated as the amino acid makeup and the PTC inhibitors have much to do with their function.
- How the ribosome can synthesize a polypeptide fragment just several amino acids long when as PTC is occupied by a large antibiotic molecule, while some others can be incorporated successfully with the synthesis is blocked in others.

HOW CAN WE MAKE CLINICAL DECISIONS REGARDING RIBOSOMAL INHIBITION?

- OBJECTIVE DATA FROM THE BUILDING, BEFORE AND AFTER VACUUM
  - FAB COMPARED TO IADAPTAIR; QUAT, SKIN CONTAM
  - EXTRACELLULAR VESICLES PCR; GENIE
- OBJECTIVE DATA FROM THE BUILDING, BEFORE AND AFTER I ADAPTAIR
- DATA FROM THE SKIN BIOME BEFORE AND AFTER Rx. ROLE OF SKIN GEL EDTA/WHISOBAX SPEED GEL (HOPKINTON)
- OBJECTIVE DATA FROM EXTRACELLULAR VESICLES, GENIE BEFORE AND AFTER PCR

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- KAROLINA MUNTER, STUDY COORDINATOR
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STUDY FUNDING

- 1 VACUUM SAMPLES EBI
- 2 IADAPT AIR SAMPLINGS EBI
- 4 SKIN MICROBIOME SAMPLINGS EBI
- 4 EXTRACELLULAR VESICLES EBI
- 5 GENIES, 10 TUBES PROGENEDX
- MATERIALS, POSTAGE, EBI TESTING STUDY REIMBURSE
- 1 DRY ICE SAMPLE MAILING STUDY REIMBURSE

DATA ACCUMULATION

- VCS BEFORE AND AFTER SAMPLING
- INTERVENTION WITH WELCHOL, BEGINNING AFTER FIRST VACUUM
- SYMPTOM ROSTER BEFORE AND AFTER SAMPLING
- PARKINSON'S SYMPTOMS BEFORE AND AFTER EACH STEP
- SKIN MICROBIOTA TIMES 4, 2 IADAPT AND 2 QUATS
- EXTRACELLULAR VESICLES BY PCR TIMES 4
- GENIE AFTER VACUUM, IADAPTAIR TIMES 2 AND AFTER QUATS TIMES 2

ANECDOTAL PARKINSON'S

ACTINO SCORE	VAC	DI	PI	QUALITY
26	IADAPT	2.9	9.2	40
18	IADAPT	1.4	15	60
20	QUAT	2.1	12.1	75
12	QUAT	1.1	6.4	90

ANECDOTAL ACTINOBACTERIA

ACTINO SCORE	VAC	DI	PI	QUALITY
27	IADAPT	1.9	43.0	10
16	QUAT 1	3.3	88.2*	25
7	QUAT 2	2.2	19.4	66
6	QUAT 3	2.0	4.1	80

POSSIBLE ACTINO/PARKINSON'S-1

- AGING, BUT WHAT ABOUT NEURAL INJURY IN YOUNG
- GUT DYSBIOSIS=ACTINOS, UNEXPLORED
- M TOR; RAPAMYCIN
- ROTENONE NEUROTOXICITY BLOCKED BY NOSCAPINE

  MMP9 BLOCKED BY NOSCAPINE
- PEP-1 RIBOSOMAL PROTEIN S3 PROTECTS DOPAMINERGIC NEURONS IN A MOUSE MODEL

  ANTIAPOPTOSIS

  ROS BLOCKADE

SEE BIBLIOGRAPHY

POSSIBLE ACTINO/PARKINSON'S-2

- NON-MOTOR SYMPTOMS CORRECTED BY INHIBITION OF MTORC1
- LOSS OF PINK1 BLOCKS HIF-1a in hypoxia
- HSP INHIBITOR ALLEVIATES a-SYNUCLEIN TOXICITY
- MAPK MODULATES ATAXIN 1
- INHIBITION OF PKR PROTECTS AGAINST TUNICAMYCIN-INDUCED APOPTOSIS
- INACTIVATION OF AKT SUPPRESSES MTOR PD PATHWAYS LEADING TO NEURONAL CELL DEATH IN PD

POSSIBLE ACTINO/PARKINSON'S-3

- STAUROSPORIN INDUCED DOPAMINERGIC OUTGROWTH FROMMMTOR SIGNALLING PATHWAYS
- DESTRUCTION OF MIDBRAIN DOPAMINERGIC NEURONS BY IMMUNOTOXIN THAT TARGETS RIBOSOME INACTIVATING PROTEIN
- BRAAK'S HYPOTHESIS APPLIES TO PD IN EARLY AGE
- SYNUCLEIN BEGIN IN OLFACTORY NERVE AND VAGUS,

LEADING TO DUAL PATHWAY OF SPREAD TO CNS FROM PERIPHERAL SOURCES

SUMMARY-1

- THERE ARE A VAST NUMBER OF BIOACTIVE SMALL MOLECULAR WEIGHT RIBOSOME INHIBITORS
- MANY ARE MANUFACTURED BY ACTINOBACTERIA
- CLINICAL STUDIES DEMONSTRATE PRESENCE OF ACTINOBACTERIA IN WDB
- REMOVAL OF ACTINOS FROM WDB PERMITS SUCCESSFUL TREATMENT OF ACUTE ACTINO-MEDIATED ILLLNESS
- NEURODEGENERATIVE PROCESSES ARE DENSELY REPRESENTED IN WDB-ACTINO ILLNESSES

SUMMARY-2

- FINE, RESTING PARKINSON-LIKE TREMOR CAN BE SHOWN AT THE BEDSIDE IN OVER 75% OF CIRS CASES
- LESS THAN 10% IN CONTROLS
- DIAGNOSTIC AND THERAPEUTIC PROTOCOLS FOCUSING ON ACTINOS MAY EXTEND TO CORRECTION OF RIBOSOMAL NEURONAL INJURY CAUSED BY ACTINOBACTERIA
- FIXING PARKINSON'S SYNDROME IS A GOOD IDEA

THANKS TO

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