Lab1

February 8, 2024

1 Lab 1

[ ]:
```
import pandas as pd
import matplotlib.pyplot as plt
import numpy as np

from sklearn.linear_model import LinearRegression
from sklearn.metrics import mean_squared_error, mean_absolute_error
from sklearn.model_selection import train_test_split
```

1.1 Part 1

1.1.1 1. Import the cell cycle dataset excel spreadsheet (using Pandas). You may need to do some tidying of the data such as dropping rows with missing NaN values.

[ ]:
```
file_path = "Cell-Cycle-Set.xlsx"
df = pd.read_excel(file_path)
df = df.dropna()

print(df.info())
```

```
<class 'pandas.core.frame.DataFrame'>
Index: 397 entries, 1 to 498
Data columns (total 10 columns):
 #   Column           Non-Null Count  Dtype
---  ------           --------------  -----
 0   Gene_Name        397 non-null    object
 1   mean_RNA_G1      397 non-null    float64
 2   mean_RNA_S       397 non-null    float64
 3   mean_RNA_G2      397 non-null    float64
 4   mean_protein_G1  397 non-null    float64
 5   mean_protein_S   397 non-null    float64
 6   mean_protein_G2  397 non-null    float64
 7   GOBP             397 non-null    object
 8   GOMF             397 non-null    object
 9   GOCC             397 non-null    object
dtypes: float64(6), object(4)
```

```
memory usage: 34.1+ KB
None
```

1.1.2 2. Do some exploratory data analysis by:

Calculate the variance and mean of the protein and mRNA concentrations

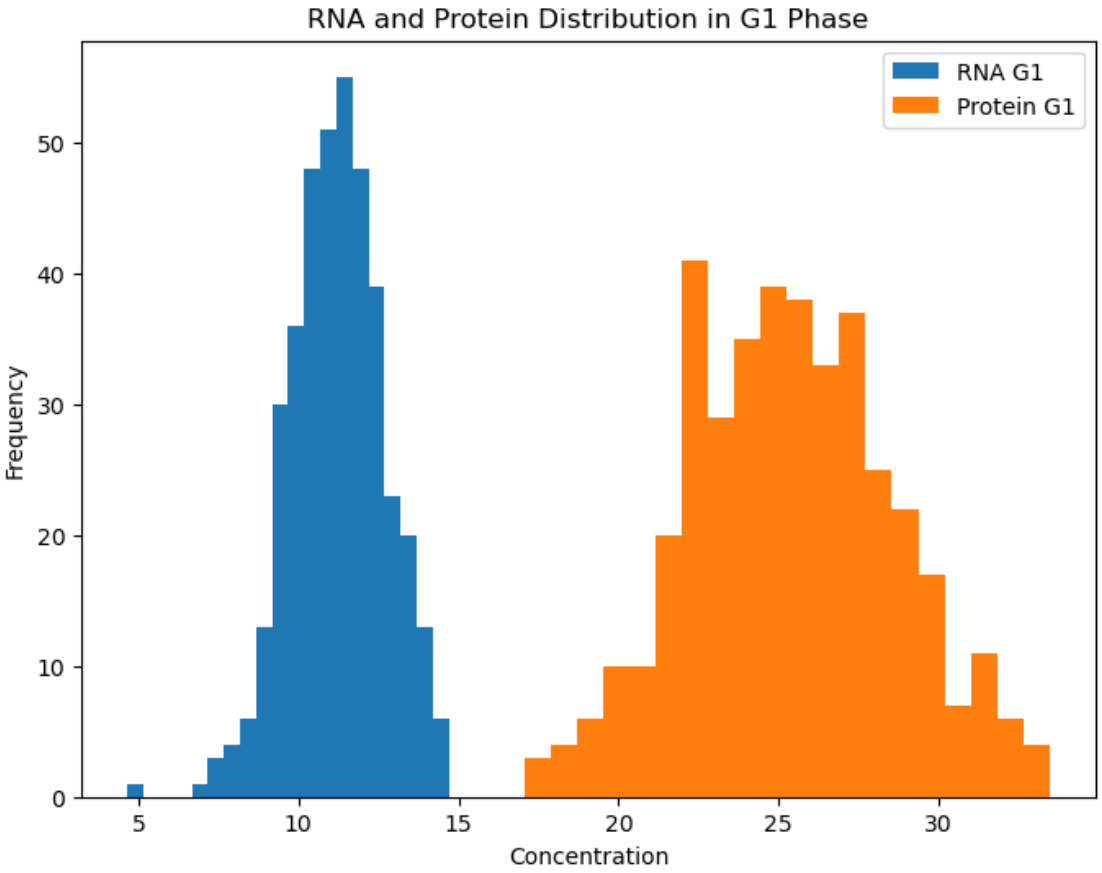
[ ]:
```
# Calculate variance and mean of RNA and protein concentrations
rna_columns = ['mean_RNA_G1', 'mean_RNA_S', 'mean_RNA_G2']
protein_columns = ['mean_protein_G1', 'mean_protein_S', 'mean_protein_G2']

variance_rna = df[rna_columns].var()
variance_protein = df[protein_columns].var()

mean_rna = df[rna_columns].mean()
mean_protein = df[protein_columns].mean()

print("Variance of RNA concentrations:")
print(variance_rna)
print("\nVariance of Protein concentrations:")
print(variance_protein)

print("\nMean of RNA concentrations:")
print(mean_rna)
print("\nMean of Protein concentrations:")
print(mean_protein)
```

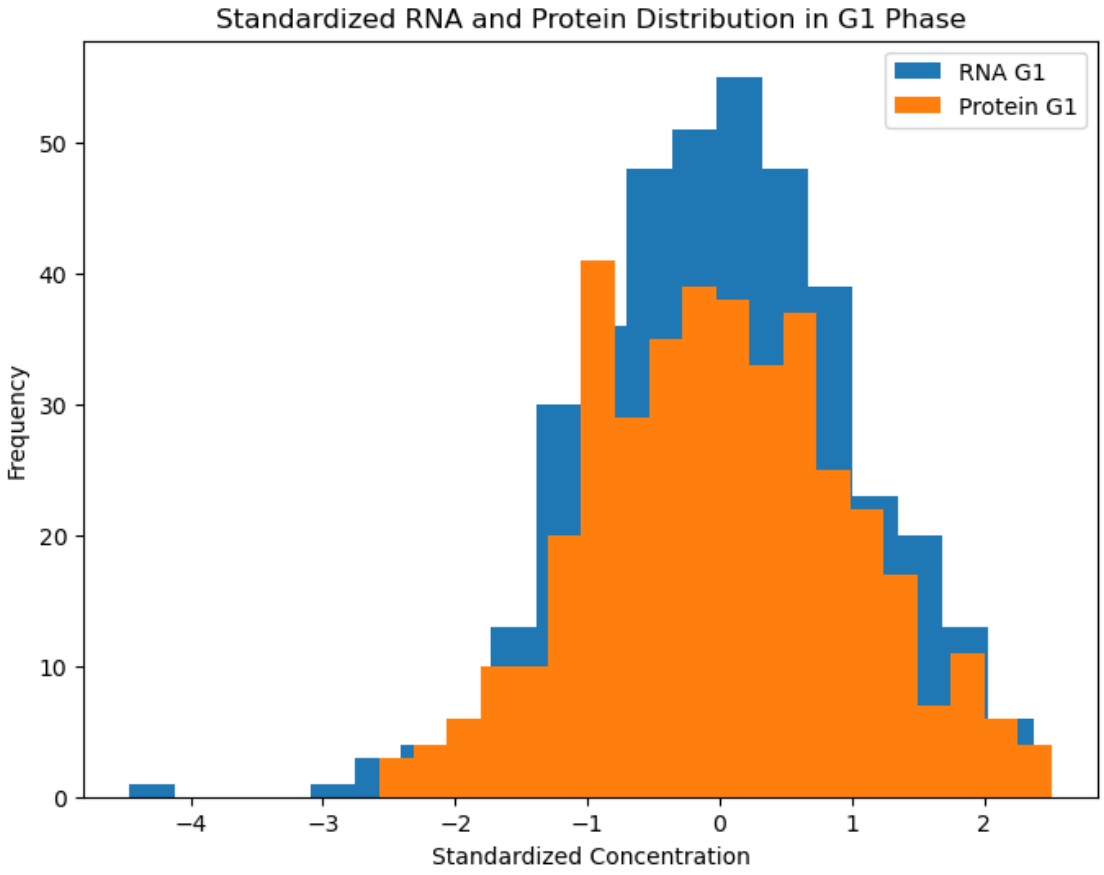
```
Variance of RNA concentrations:
mean_RNA_G1    2.160506
mean_RNA_S     2.145592
mean_RNA_G2    2.101649
dtype: float64

Variance of Protein concentrations:
mean_protein_G1    10.453574
mean_protein_S     10.403018
mean_protein_G2    10.018217
dtype: float64

Mean of RNA concentrations:
mean_RNA_G1    11.215627
mean_RNA_S     11.186962
mean_RNA_G2    11.257939
dtype: float64

Mean of Protein concentrations:
mean_protein_G1    25.351672
mean_protein_S     22.847658
mean_protein_G2    25.573553
```

```
dtype: float64
```

Generate a histogram of one of the cell cycle stages of the RNA and protein distribution

[ ]:
```
plt.figure(figsize=(8, 6))
plt.hist(df['mean_RNA_G1'], bins=20, label='RNA G1')
plt.hist(df['mean_protein_G1'], bins=20, label='Protein G1')
plt.title('RNA and Protein Distribution in G1 Phase')
plt.xlabel('Concentration')
plt.ylabel('Frequency')
plt.legend()
plt.show()

# Standardize concentrations
std_rna = (df[rna_columns] - mean_rna) / df[rna_columns].std()
std_protein = (df[protein_columns] - mean_protein) / df[protein_columns].std()

plt.figure(figsize=(8, 6))
plt.hist(std_rna['mean_RNA_G1'], bins=20, label='RNA G1')
plt.hist(std_protein['mean_protein_G1'], bins=20, label='Protein G1')
plt.title('Standardized RNA and Protein Distribution in G1 Phase')
plt.xlabel('Standardized Concentration')
plt.ylabel('Frequency')
plt.legend()
plt.show()
```


RNA and Protein Distribution in G1 Phase
RNA G1
Protein G1
Frequency
Concentration
0
10
20
30
40
50
5
10
15
20
25
30



State what you notice about these, and how this might affect inferring Protein from mRNA concentrations From the histogram above, it is noticable that the concentration of the protein is signicantly higher than the concentration of the RNA.

Typically, a single mRNA is used to synthesise multiple copies of the corresponding protein which could explain why the concentration level of the protein is significantly higher than the RNA. Specifically in the G1 phase, cell growth is occuring where proteins are synthesised to prepare for the S phase.

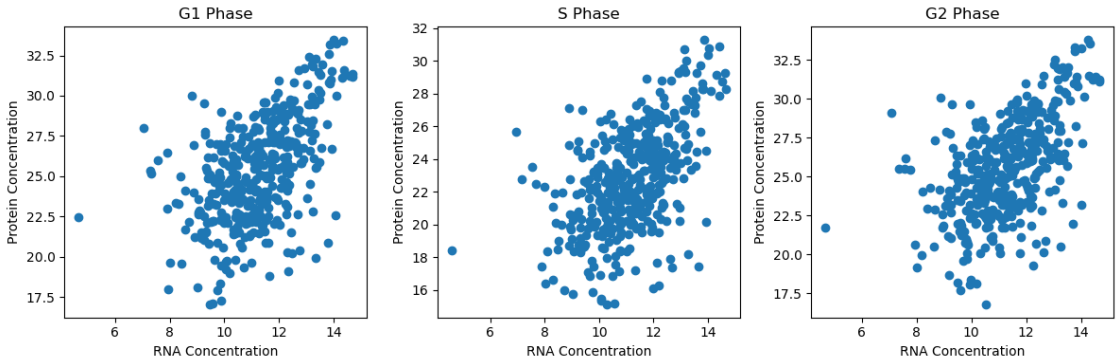
With this we can identify some relationship between protein and mRNA concentrations because as mRNA concentration increases, the potential for protein synthensis to occur is higher.

Generate a scatterplot of the RNA vs Protein concentrations for each stage of the cell cycle.

[ ]:
```
stages = ['G1', 'S', 'G2']
```

[ ]:
```
fig, axes = plt.subplots(1, 3, figsize=(12, 4))

for i, stage in enumerate(stages):
    axes[i].scatter(df[f'mean_RNA_{stage}'], df[f'mean_protein_{stage}'])
```

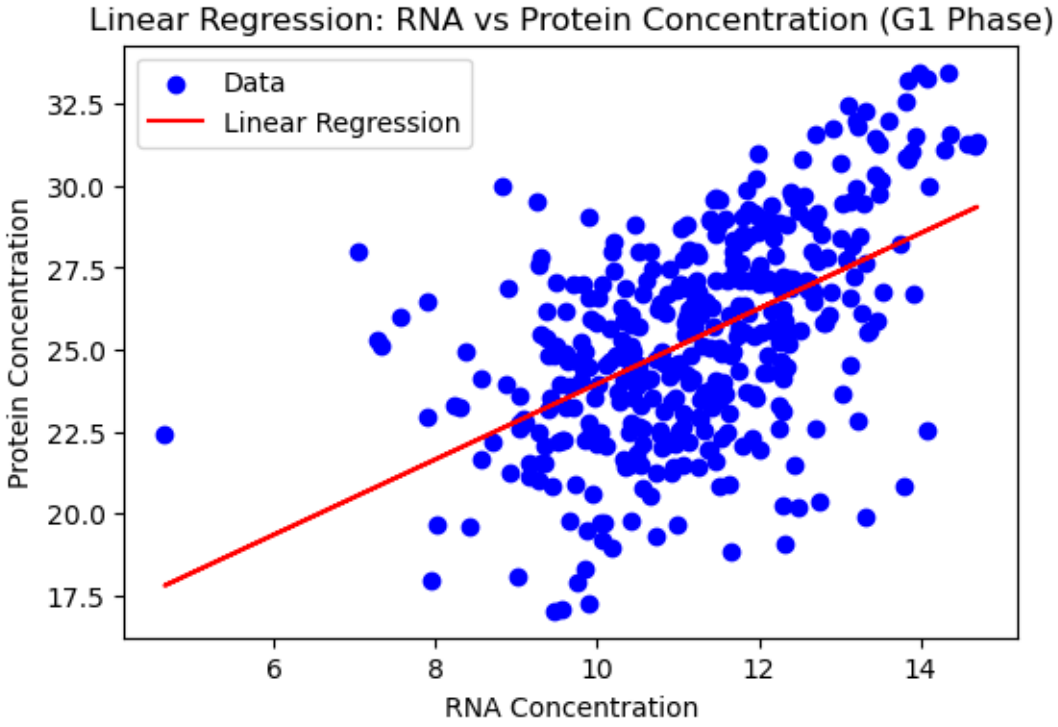
```
    axes[i].set_title(f'{stage} Phase')
    axes[i].set_xlabel('RNA Concentration')
    axes[i].set_ylabel('Protein Concentration')

plt.tight_layout()
plt.show()
```



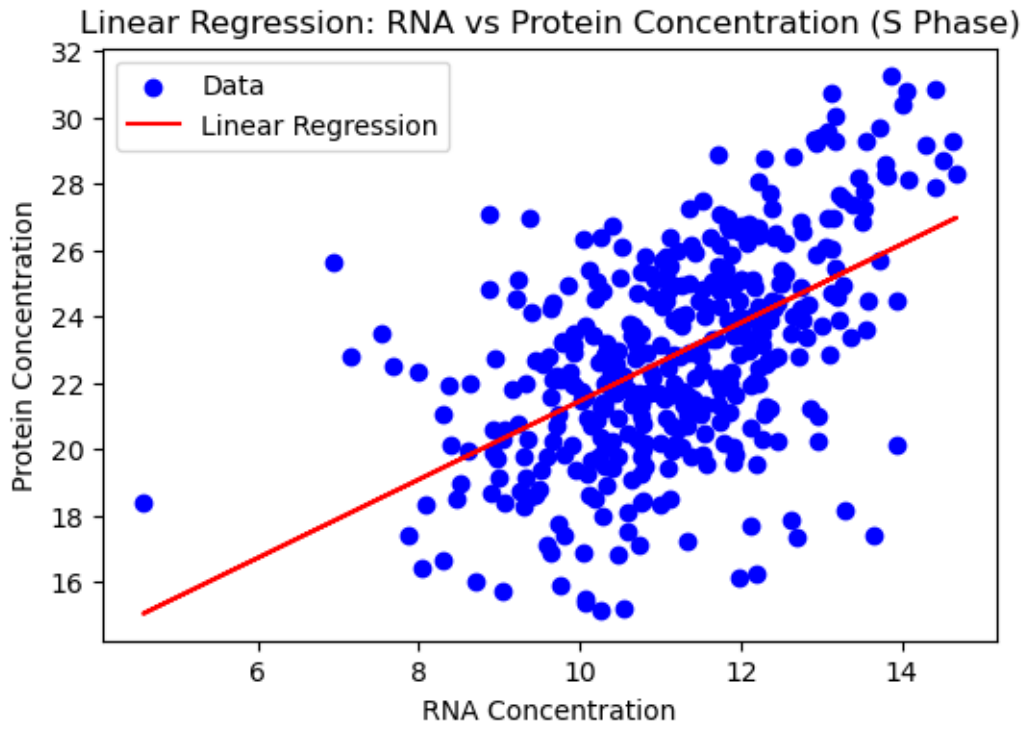
Fit a linear model (use sklearn or your own direct solve of linear regression)

[ ]:
```
for stage in stages:
    X = df[f'mean_RNA_{stage}'].values.reshape(-1, 1)
    y = df[f'mean_protein_{stage}']

    # X_train, X_test, y_train, y_test = train_test_split(X, y, test_size=0.2,␣
 ↪random_state=42)

    linear = LinearRegression()
    linear.fit(X, y)

    y_pred = linear.predict(X)

    rmse = np.sqrt(mean_squared_error(y, y_pred))

    print(f'{stage} Phase RMSE: {rmse}')

    plt.figure(figsize=(6, 4))
    plt.scatter(X, y, color='blue', label='Data')
    plt.plot(X, y_pred, color='red', label='Linear Regression')
    plt.title(f'Linear Regression: RNA vs Protein Concentration ({stage}␣
 ↪Phase)')
    plt.xlabel('RNA Concentration')
    plt.ylabel('Protein Concentration')
    plt.legend()
    plt.show()
```

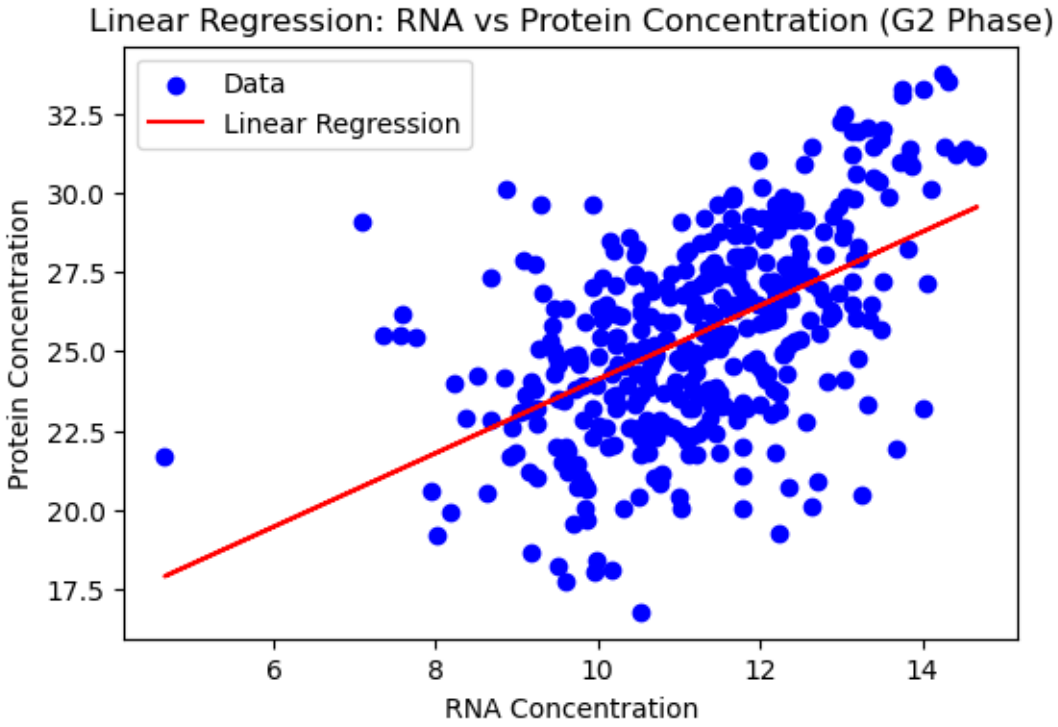
```
G1 Phase RMSE: 2.7529658342371697
```




```
S Phase RMSE: 2.719095472683103
```




```
G2 Phase RMSE: 2.6755780028716756
```



How accurate would predicitons of Protein concentration be using just RNA concentration? Can you quantify this? To calculate the accuracy of our linear regression model, we can make use of RMSE. On average, an RMSE of 2.716 was achieved in the three phases. This means that on average we are 2.716 units away from an accurate protein prediction given an RNA. As the range of the protein concentration is around 17-33, an RMSE of 2.716 might be acceptable but is not completely accurate.

The large difference in variance in protein concentration (10.292) and RNA concentration (2.136) could explain why our predictions are not the best and that predicting the protein concentration might be more challenging and not as straight foreward as a direct correlation.

Furthermore, by looking at the scatter plots of the data we can see this large variance in protein concentration which may require a more complex model than a linear one.

1.2 Part 2

1.2.1 Find all genes that contain 'cell cycle' in their GOBP term and plot them as a scatterplot (with different colour) overlaid across all genes for each cell cycle phase.

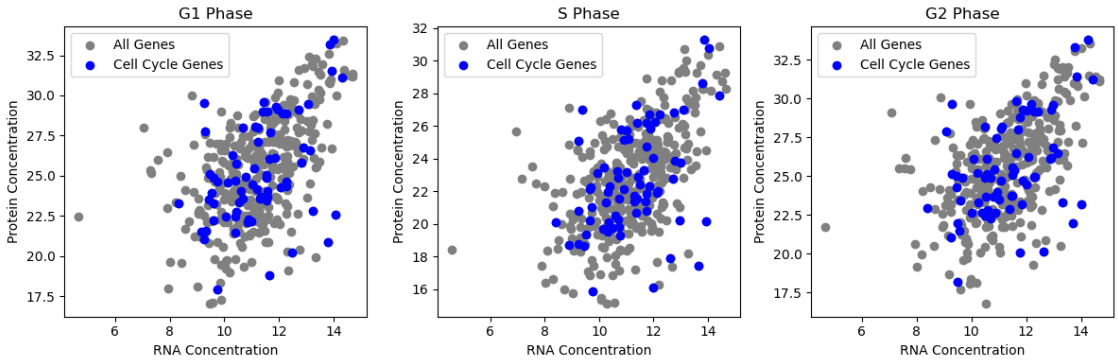
Calculate the correlations.

Comment on how these compare, link this to your understanding of the Cell Cycle

[ ]:
```
cell_cycle_GOBP = df[df.GOBP.str.contains('cell cycle', case = False)]

fig, axes = plt.subplots(1, 3, figsize=(12, 4))

for i, stage in enumerate(stages):
    axes[i].scatter(df[f'mean_RNA_{stage}'], df[f'mean_protein_{stage}'],␣
 ↪color='gray', label='All Genes')
    axes[i].scatter(cell_cycle_GOBP[f'mean_RNA_{stage}'],␣
 ↪cell_cycle_GOBP[f'mean_protein_{stage}'], color='blue', label='Cell Cycle␣
 ↪Genes')
    axes[i].set_title(f'{stage} Phase')
    axes[i].set_xlabel('RNA Concentration')
    axes[i].set_ylabel('Protein Concentration')
    axes[i].legend()

    stage_corr = df[f'mean_RNA_{stage}'].corr(df[f'mean_protein_{stage}'])
    print(f'{stage} phase RNA-Protein Correlation: {stage_corr}')

    stage_corr = cell_cycle_GOBP[f'mean_RNA_{stage}'].
 ↪corr(cell_cycle_GOBP[f'mean_protein_{stage}'])
    print(f'{stage} phase "cell cycle" RNA-Protein Correlation: {stage_corr}\n')
```

```
plt.tight_layout()
plt.show()
```

```
G1 phase RNA-Protein Correlation: 0.522657733063862
G1 phase "cell cycle" RNA-Protein Correlation: 0.4365460497902055

S phase RNA-Protein Correlation: 0.5361902686743045
S phase "cell cycle" RNA-Protein Correlation: 0.43838828414419223

G2 phase RNA-Protein Correlation: 0.5325650185250105
G2 phase "cell cycle" RNA-Protein Correlation: 0.4533272550044794
```



The correlation of the RNA and protein concentration at each phase for genes related to cell cycle is positive but weak. The 'cell cycle' correlation is also lower than the overall correlation which could indicate that genes related to the cell cycle are more complex.

1.2.2 Find all genes that contain 'ribosome' in their GOCC term and plot them as a scatterplot (with different colour) overlaid across all genes for each cell cycle phase.

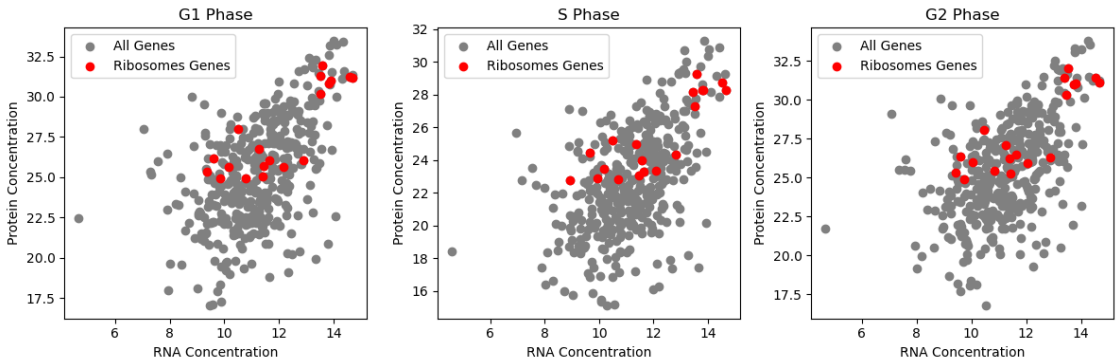
Calculate the correlations.

Comment on how these compare, link this to your understanding of the Cell Cycle.

[ ]:
```
cell_cycle_GOCC = df[df.GOCC.str.contains('ribosome', case = False)]

fig, axes = plt.subplots(1, 3, figsize=(12, 4))

for i, stage in enumerate(stages):
    axes[i].scatter(df[f'mean_RNA_{stage}'], df[f'mean_protein_{stage}'],␣
 ↪color='gray', label='All Genes')
    axes[i].scatter(cell_cycle_GOCC[f'mean_RNA_{stage}'],␣
 ↪cell_cycle_GOCC[f'mean_protein_{stage}'], color='red', label='Ribosomes␣
 ↪Genes')
```

```
    axes[i].set_title(f'{stage} Phase')
    axes[i].set_xlabel('RNA Concentration')
    axes[i].set_ylabel('Protein Concentration')
    axes[i].legend()

    stage_corr = df[f'mean_RNA_{stage}'].corr(df[f'mean_protein_{stage}'])
    print(f'{stage} phase RNA-Protein Correlation: {stage_corr}')

    stage_corr = cell_cycle_GOCC[f'mean_RNA_{stage}'].
↪corr(cell_cycle_GOCC[f'mean_protein_{stage}'])
    print(f'{stage} phase "ribosome" RNA-Protein Correlation: {stage_corr}\n')

plt.tight_layout()
plt.show()
```

```
G1 phase RNA-Protein Correlation: 0.522657733063862
G1 phase "ribosome" RNA-Protein Correlation: 0.8408005925694083

S phase RNA-Protein Correlation: 0.5361902686743045
S phase "ribosome" RNA-Protein Correlation: 0.8448011378787453

G2 phase RNA-Protein Correlation: 0.5325650185250105
G2 phase "ribosome" RNA-Protein Correlation: 0.8477056210062089
```



The correlation of the RNA and protein concentration at each phase for genes related to ribosomes is very strong. This indicates that there is a stronger relationship between the two.

This aligns with our understanding of the cell cycle as we know that ribosomes play a crucial role in protein synthesis. Ribosomes decode the genetic information from the mRNA to synthesise proteins thus if there is an increase in mRNA concentration, then protein concentration will likely increase to a similar degree.

1.2.3 Count the number of occurrences of every GOBP term across all genes, what are some of the difficulties that arise when using these terms?

[ ]:
```
print(df.GOBP.str.split(";").explode().value_counts())
```

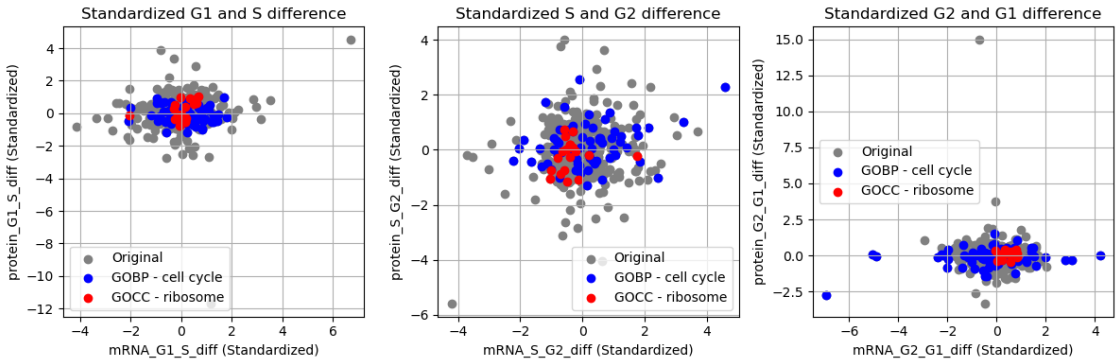
```
GOBP
cellular process                    377
metabolic process                   273
cellular metabolic process          260
primary metabolic process           255
biological regulation               236
                                   ...
rRNA import into mitochondrion        1
RNA import into mitochondrion         1
cyanate metabolic process             1
cyanate catabolic process             1
response to interferon-alpha          1
Name: count, Length: 2854, dtype: int64
```

One difficulty we may face is ambiguity and redundancy. Many terms encompass one another (e.g. cellular component organization or biogenesis at cellular level and cellular component organization at cellular level) and it might become difficult to distinguish them from eachother.

1.2.4 Calculate the change in mRNA/protein levels across the cell cycle by taking the difference at each stage (G1-S, S-G2, G2-G1), and standardize the differences by mean-centering and variance scaling.

[ ]:
```
# Calculate differences in mRNA and protein levels across cell cycle stages
df['mRNA_G1_S_diff'] = df['mean_RNA_S'] - df['mean_RNA_G1']
df['mRNA_S_G2_diff'] = df['mean_RNA_G2'] - df['mean_RNA_S']
df['mRNA_G2_G1_diff'] = df['mean_RNA_G1'] - df['mean_RNA_G2']

df['protein_G1_S_diff'] = df['mean_protein_S'] - df['mean_protein_G1']
df['protein_S_G2_diff'] = df['mean_protein_G2'] - df['mean_protein_S']
df['protein_G2_G1_diff'] = df['mean_protein_G1'] - df['mean_protein_G2']

df.iloc[:,-6:] = (df.iloc[:,-6:] - df.iloc[:,-6:].mean(axis=0)) / df.iloc[:,-6:
 ↪].std(axis=0)

cell_cycle_GOBP = df[df.GOBP.str.contains('cell cycle', case=False)]
cell_cycle_GOCC = df[df.GOCC.str.contains('ribosome', case=False)]

fig, axes = plt.subplots(1, 3, figsize=(12, 4))

axes[0].scatter(df['mRNA_G1_S_diff'], df['protein_G1_S_diff'], color='grey',␣
 ↪label='Original')
```

```
axes[0].scatter(cell_cycle_GOBP['mRNA_G1_S_diff'],␣
 ↪cell_cycle_GOBP['protein_G1_S_diff'], color='blue', label='GOBP - cell␣
 ↪cycle')
axes[0].scatter(cell_cycle_GOCC['mRNA_G1_S_diff'],␣
 ↪cell_cycle_GOCC['protein_G1_S_diff'], color='red', label='GOCC - ribosome')
axes[0].set_xlabel('mRNA_G1_S_diff (Standardized)')
axes[0].set_ylabel('protein_G1_S_diff (Standardized)')
axes[0].set_title('Standardized G1 and S difference')
axes[0].legend()
axes[0].grid(True)

axes[1].scatter(df['mRNA_S_G2_diff'], df['protein_S_G2_diff'], color='grey',␣
 ↪label='Original')
axes[1].scatter(cell_cycle_GOBP['mRNA_S_G2_diff'],␣
 ↪cell_cycle_GOBP['protein_S_G2_diff'], color='blue', label='GOBP - cell␣
 ↪cycle')
axes[1].scatter(cell_cycle_GOCC['mRNA_S_G2_diff'],␣
 ↪cell_cycle_GOCC['protein_S_G2_diff'], color='red', label='GOCC - ribosome')
axes[1].set_xlabel('mRNA_S_G2_diff (Standardized)')
axes[1].set_ylabel('protein_S_G2_diff (Standardized)')
axes[1].set_title('Standardized S and G2 difference')
axes[1].legend()
axes[1].grid(True)

axes[2].scatter(df['mRNA_G2_G1_diff'], df['protein_G2_G1_diff'], color='grey',␣
 ↪label='Original')
axes[2].scatter(cell_cycle_GOBP['mRNA_G2_G1_diff'],␣
 ↪cell_cycle_GOBP['protein_G2_G1_diff'], color='blue', label='GOBP - cell␣
 ↪cycle')
axes[2].scatter(cell_cycle_GOCC['mRNA_G2_G1_diff'],␣
 ↪cell_cycle_GOCC['protein_G2_G1_diff'], color='red', label='GOCC - ribosome')
axes[2].set_xlabel('mRNA_G2_G1_diff (Standardized)')
axes[2].set_ylabel('protein_G2_G1_diff (Standardized)')
axes[2].set_title('Standardized G2 and G1 difference')
axes[2].legend()
axes[2].grid(True)

plt.tight_layout()
plt.show()
```



1.2.5 What do we notice about changes in the cell cycle? Is there any apparent clustering of GO terms?

In the scatter plots above, we can see that there are typically only slight changes in RNA and protein concentration. We can also see that the data points containing 'ribosome' in the GOCC label are clustered together and show the least amount of change between each stage compared to the overall data. Regarding genes containing 'cell cycle' in the GOBP label, there doesn't seem to be any clustering different from the overall dataset.

1.2.6 Continue the exploration using other terms you have met in the lectures, try to find clusters or correlations