A culture of mammalian cells was first treated with nocodazole (which antagonizes microtubule formation and hence arrests cells at the beginning of mitosis) for 24 hours to synchronize the cell population. The nocodazole was then removed from the cell culture and the culture was then continuously incubated with \[^3\text{H}\]-thymidine for the next 48 hours. The amount of incorporation of \[^3\text{H}\]-thymidine into the cells was plotted as a function of time after the start of incubation with \[^3\text{H}\]-thymidine. These results are shown in the figure below.

a) In the above figure, what does the increase in the amount of incorporated \[^3\text{H}\]-thymidine into DNA starting at 10 hours represent?

b) Assuming that the mitotic division period, \(M\), equals 1 hour, determine the following cell-cycle parameters based on the figure above.

i) The length of the total cell cycle period______
ii) The length of the G1 phase_____

iii) The length of the S phase__________

iv) The length of the G2 phase_____

Part B
When studying cell division cycle (cdc) mutants, you can use a series of reciprocal shift experiments to determine where in the cell cycle these temperature sensitive mutants are blocked. One example is outlined below.

Condition 1) Incubate cdcX^- yeast at 20°C with hydroxyurea present until all the cells are synchronized in S phase; you then shift the cells to 36°C and remove the hydroxyurea. You then monitor the cells to determine if they complete mitosis during the second incubation.

Condition 2) Incubate cdcX^- yeast at 37°C; you then add hydroxyurea and shift the cells to 20°C. You then monitor the cells to determine if they complete mitosis during the second incubation.

a) Complete the table below.

<table>
<thead>
<tr>
<th>Block point</th>
<th>Completion of mitosis during second incubation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Condition 1</td>
</tr>
<tr>
<td>yes</td>
<td>yes</td>
</tr>
<tr>
<td>no</td>
<td>no</td>
</tr>
<tr>
<td>no</td>
<td>no</td>
</tr>
</tbody>
</table>

b) If you were to screen temperature-sensitive yeast mutants using the above reciprocal shift experiments, what observed block point would you predict for the following?

Cells with a mutation in the gene encoding the G2 cyclin.

Cells with a mutation in the gene encoding the G1 cyclin.

Cells with a mutation in the gene encoding the cdk2 kinase subunit.

Cells with two mutations, in genes encoding both the G1 cyclin and the G2 cyclin.