Evidence for a $\gamma$-GT independent pathway for GSH degradation
4.1 Introduction:

γ-Glutamyl transpeptidase (γ-GT), (as has been discussed earlier) catalyzes the first step in the degradation of glutathione which involves the cleavage of γ-glutamyl moiety and release of cysteinylglycine. Cysteinylglycine in turn is further hydrolyzed into constituent amino acids, cysteine and glycine, by a still unidentified gene (in yeasts) coding for the cysteinylglycine dipeptidase. So far, γ-GT is the only enzyme known to be involved in the degradation and turnover of glutathione in all living systems (Meister et al., 1981; Jaspers et al., 1985).

The possibility that in addition to γ-GT, a second degradation pathway might exist in living cells is an aspect of glutathione homeostasis that has not been properly addressed. The recent identification of the ECM38 gene in the yeast S. cerevisiae as encoding the γ-GT enzyme, and the ease of genetic manipulation in yeast offers several possibilities for investigating these questions.

In the present chapter we describe our efforts to investigate the regulation of γ-GT at the transcriptional level by nitrogen sources, sulphur sources and GSH. Subsequently we have investigated the question as to whether glutathione turnover and degradation can occur even in the absence of γ-GT. These latter studies were carried out by examining how the presence or absence of γ-GT affects growth of yeasts and total GSH levels in strain backgrounds where endogenous synthesis of glutathione does not occur. We have also investigated this issue using a met15Δ strain requiring glutathione (or other organic sulphur sources) as a source of exogenous sulphur. The results of these investigations are summarized in this chapter.

4.2 Transcriptional regulation of the ECM38 gene:

To initiate studies on the roles of γ-GT, it was first important to determine under what conditions the enzyme is regulated. Previous studies (at the enzyme activity level) have indicated reduced γ-GT enzyme activity in cells grown in ammonium (Jaspers et al., 1985). Further it has also been shown that γ-GT is derepressed in gsh1Δ strains, which was most probably due to an alteration of the endogenous thiol status (Elskens et al., 1991). We decided to undertake a more detailed investigation on the regulation of γ-GT enzyme, by examining the transcriptional regulation of ECM38, to get some further insights into it's true roles within the cell. Studies on the regulation of ECM38 gene were carried out using promoter-β-gal fusions, where 634 bp of the ECM38 gene
was fused in frame with lacZ in the multicopy vector pLG699Z. Studies with this promoter-reporter fusion indicated that ECM38 was primarily under nitrogen regulation, and that growth in derepressing nitrogen sources (such as glutamate) led to a very significant induction in activity (Table 4.1). Relatively milder repression was observed with methionine. In the presence of glutathione, we did not find any increase or decrease in β-gal activity. The fact that ECM38 is not regulated transcriptionally by glutathione is interesting, but perhaps not surprising, considering that other genes involved in glutathione biosynthesis, such as GSH1 are also not regulated by GSH levels (Stephen and Jamieson, 1997). As the γ-GT enzyme has been earlier shown to localize to the yeast vacuole (Jaspers and Penninckx, 1984), the β-gal reporter studies described above suggest that the enzyme has a specialized role for utilizing vacuolar stores of glutathione during nitrogen starvation (and to a lesser extent, sulphur starvation) conditions (Elskens et al., 1991; Mehdi and Penninckx, 1997).

The expression of most nitrogen catabolic genes in Saccharomyces cerevisiae is regulated at the level of transcription in response to the quality of nitrogen source available. This regulation is accomplished through four GATA-family transcription factors: two positively acting factors capable of transcriptional activation (Gln3p and Gat1p) and two negatively acting factors capable of down-regulating Gln3p- and/or Gat1p-dependent transcription (Dal80p and Deh1p) (Coffman and Cooper, 1997). These regulatory proteins bind to motifs in the promoter region having consensus sequence 5' GATA 3'. In addition to the above four GATA-family transcription factors, nitrogen catabolic gene expression is negatively regulated by Ure2p and one or more unidentified proteins which appear to function analogously (Courchesne and Magasanik, 1988; Hoffman-Bang, 1999). We thought it worth examining if γ-GT is also under nitrogen catabolite repression mediated by these factors. When we examined expression in an gln3Δ strain the activity was significantly reduced even in glutamate medium, and in an ure2Δ strain, the activity was constitutively activated even in ammonium sulphate medium, indicating that the genes were under the classical nitrogen catabolite repression (Table 4.1).
TABLE 4.1 Transcriptional Regulation of ECM38 under different growth conditions and genetic backgrounds

<table>
<thead>
<tr>
<th>Strains</th>
<th>Growth Conditions</th>
<th>(\beta)-Galactosidase activity (units of (\beta)-gal/OD(_{600}) cells)</th>
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</thead>
<tbody>
<tr>
<td>(P_{ECM38})-lac(Z)/ABC734 (BY4742)</td>
<td>SD + ammonium sulphate</td>
<td>1.9 ± 0.2</td>
</tr>
<tr>
<td>(P_{ECM38})-lac(Z)/ABC734 (BY4742)</td>
<td>SD + glutamate</td>
<td>17.0 ± 2.3</td>
</tr>
<tr>
<td>(P_{ECM38})-lac(Z)/ABC734 (BY4742)</td>
<td>SD + glutamate + methionine</td>
<td>5.5 ± 0.7</td>
</tr>
<tr>
<td>(P_{ECM38})-lac(Z)/ABC734 (BY4742)</td>
<td>SD + glutamate + cysteine</td>
<td>11.8 ± 1.5</td>
</tr>
<tr>
<td>(P_{ECM38})-lac(Z)/ABC734 (BY4742)</td>
<td>SD + glutamate + glutathione</td>
<td>12.2 ± 2.1</td>
</tr>
<tr>
<td>(P_{ECM38})-lac(Z)/ABC1094 (gln3(\Delta))</td>
<td>SD + ammonium sulphate</td>
<td>0.4 ± 0.1</td>
</tr>
<tr>
<td>(P_{ECM38})-lac(Z)/ABC1094 (gln3(\Delta))</td>
<td>SD + glutamate</td>
<td>0.4 ± 0.1</td>
</tr>
<tr>
<td>(P_{ECM38})-lac(Z)/ABC1113 (ure2(\Delta))</td>
<td>SD + ammonium sulphate</td>
<td>7.2 ± 3.4</td>
</tr>
<tr>
<td>(P_{ECM38})-lac(Z)/ABC1113 (ure2(\Delta))</td>
<td>SD + glutamate</td>
<td>21.4 ± 9.0</td>
</tr>
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</table>

\(P_{ECM38}\)-lac\(Z\): ECM38 promoter cloned from ABC734 (BY4742)
4.3 Overexpression of γ-GT in yeast restores glutathione levels in strains deleted in ECM38, to wild type levels, but does not lead to enhanced GSH turnover rates:

To investigate the role of γ-GT in GSH homeostasis, we created disruptions in the GSH1 gene in a wild type strain ABC734 (BY4742) and in ABC1066 (ecm38Δ in BY4742) (gsh1Δ cells are deleted for endogenous glutathione biosynthesis). The gsh1Δ disruption was created by using a LEU2 based disruption plasmid (Materials and Methods, section 2.1.3.2). These disruption strains were used to investigate how the presence or absence of γ-GT affected the total glutathione pools. Cells were grown in glutamate medium as this is known to increase γ-GT activity. As expected, cells lacking γ-GT (gsh1Δ ecm38Δ) had an almost 3-fold higher glutathione content than the gsh1Δ cells containing γ-GT (gsh1Δ ECM38) (Table 4.2).

During earlier investigations from our lab describing the phenomenon of 'delayed growth stasis' upon GSH depletion, S. cerevisiae gsh1Δ cells were found to be able to grow for an additional 7-8 generations after transfer to glutathione-free medium before entering into growth stasis (Sharma et al., 2000). Further studies, however, revealed that the YPH499 strain background being used lacked any γ-glutamyl transpeptidase activity even when grown under conditions known to increase γ-glutamyl transpeptidase activity in the cell (discussed in detail in Chapter 3). Since γ-GT is the only enzyme known to be involved in glutathione turnover it was possible that the prolonged growth in the absence of glutathione that we were observing in YPH499 backgrounds was a result of the absence of this enzyme in this background. Since the strain background used for measuring GSH pools in the present studies was BY4742 and had a functional γ-GT (Table 3.1, Chapter 3), we re-examined the delayed growth stasis phenomenon in these strains to determine if there was an increased delayed stasis in ecm38Δ deletion strains as compared to ECM38 strains when cells were grown on ammonium or glutamate as nitrogen source. We observed that the strains, gsh1Δ ECM38 and gsh1Δ ecm38Δ, were identical in the response to glutathione deprivation irrespective of the nitrogen source in the medium (glutamate or ammonium) and showed the same 'delayed growth stasis' behavior seen in the gsh1Δ strains in the YPH499 background. The presence or absence of a functional ECM38 gene did not affect either the growth patterns or the delayed growth stasis and indicated
Table 4.2 Effect of γ-GT on the Glutathione content of different yeast strains

<table>
<thead>
<tr>
<th>Strain</th>
<th>Relevant Genotype</th>
<th>GSH levels at Time (nmoles GSH/10^8 cells)</th>
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<tr>
<td></td>
<td></td>
<td>'0'h</td>
</tr>
<tr>
<td>ABC1195</td>
<td>(gshlΔ)</td>
<td>9.4±0.3</td>
</tr>
<tr>
<td>ABC1196</td>
<td>(gshlΔ ecm38Δ)</td>
<td>23.1±2.5</td>
</tr>
<tr>
<td>ABC1196</td>
<td>(gshlΔ ecm38Δ)</td>
<td>29.2±2.8</td>
</tr>
<tr>
<td></td>
<td>(p-TEF)</td>
<td></td>
</tr>
<tr>
<td>ABC1196</td>
<td>(gshlΔ ecm38Δ)</td>
<td>9.3±0.6</td>
</tr>
<tr>
<td></td>
<td>(p-TEF-ECM38)</td>
<td></td>
</tr>
</tbody>
</table>
that the γ-GT enzyme, did not affect to any significant extent the glutathione pools responsible for cell growth.

The surprising observations described above prompted us to further investigate the role of γ-GT. We overexpressed γ-GT enzyme by expressing it downstream of a strong constitutive promoter. The p-TEF-ECM38 containing strains had many-fold higher activity of γ-GT enzyme (Table 3.1, Chapter 3) and restored the GSH pools from $23.1\pm2.5$ nM observed in ecm38Δ cells to $9.4\pm0.3$ nM, levels seen in ECM38 cells (Table 4.2). However surprisingly, the presence of an overproduced γ-GT did not display any toxicity, or decreased growth as seen from growth curve experiments (data not shown). This perhaps is a result of the fact that the cellular glutathione levels are not completely depleted even when the γ-GT levels in the cell are significantly high.

We also attempted to evaluate the glutathione turnover rates in ECM38 and ecm38Δ strains in the gsh1Δ background. Although the glutathione/cell number ratio decreases with time, this results primarily from the increase in cell number. The apparent glutathione half life determined on the basis of the glutathione content per cell OD was $136\pm7.4$ min$^{-1}$ in ECM38 cells and $132\pm13.8$ min$^{-1}$ in ecm38Δ cells. However as there is a concurrent increase in cell number with a generation time of $153\pm2.8$ min. in case of ECM38 cells while $144\pm1.4$ min. in case of ecm38Δ cells, the effective glutathione turnover is exceedingly slow or negligible in both ECM38 and ecm38Δ cells. The GSH half-life is essentially the dilution of existing pools of GSH into growing cells.

4.4 The ability of the organic sulphur auxotroph met15Δ strains to utilize glutathione as an exogenous sulphur source is independent of the γ-GT activity in the cell:

The evidence with gsh1Δ strains described above clearly indicated that the total glutathione turnover rate was negligible in these strains. We decided to examine the issue of glutathione turnover through an alternative genetic strategy in a met15Δ strain, BY4741, a strain in the BY4742 background series carrying a met15Δ. Strains defective in MET15 are organic sulphur auxotrophs as they lack the enzyme for the reduction of sulphate (Thomas et al., 1992). These strains are able to grow on methionine, glutathione or cysteine as an organic sulphur source but are unable to grow
in the absence of organic sulphur. If the presence of γ-GT was indeed mandatory for glutathione turnover and degradation in the cell, we argued that in a met15Δ ecm38Δ background, these cells would be able to grow in methionine or cysteine as a sole organic sulphur source, but not with glutathione as the sole sulphur source. We created an ecm38Δ in a met15Δ background and examined the growth of these cells in glutathione and methionine. The experiments were carried out in ammonium sulphate medium as well as in glutamate medium where γ-GT activity is known to be high. The fact that glutathione is actually transported inside through a specific glutathione transporter (Hgt1p) prior to utilization has been demonstrated earlier in our laboratory since met15Δ in an hgt1Δ background is unable to grow on GSH as sole organic sulphur source (Bourbouloux et al., 2000). Both in plates as well as in liquid medium we could not see any differences in growth (and growth rates) for the met15Δ cells as compared to the met15Δ ecm38Δ cells on glutathione (Fig 4.1 and Fig 4.2). Furthermore the growth rates were almost comparable to that on methionine and there were also no significant differences in growth rate between use of ammonium sulphate or glutamate as the nitrogen source. This is in contrast to γ-GT regulation, which is strongly regulated by the nature of the nitrogen source.

Having already shown that γ-GT is not needed for the utilization of GSH as an exogenous sole source of sulphur, we further examined if this γ-GT independent utilization of GSH could also occur in a gsh1Δ background (where GSH has an essential growth function), we introduced a deletion in the GSH1 gene in a met15Δecm38Δ background. We observed that in these gsh1Δ met15Δ ecm38Δ strains (where glutathione is essential for growth as a redox requirement and as a sulphur source), the utilization of glutathione occurs very efficiently in a manner similar to that of met15Δ ecm38Δ GSH1 strains (Fig 4.3). These results indicate that even in a gsh1Δ background efficient turnover and utilization of glutathione can occur.

We also checked whether γ-GT is needed for the utilization of γ-glutamylcysteine, a dipeptide intermediate formed during GSH biosynthesis, which has γ-glutamyl linkage. Growth of met15Δ and met15Δ ecm38Δ cells was examined on plates having γ-glutamylcysteine as the sole source of sulphur. We could not observe any differences in growth for the met15Δ cells as compared to the met15Δ ecm38Δ cells on plates having γ-glutamylcysteine (Fig 4.4). Yeast cells were able to utilize γ-
Fig 4.1 Growth of met15Δ and met15Δecm38Δ on SD plates having ammonium sulphate as the nitrogen source and (A) methionine, (B) GSH, (C) cysteine as sulphur sources respectively. (D) without added organic sulphur source. GSH, methionine and cysteine were used at a concentration of 0.25mM. Similar growth was observed when glutamate was used as the nitrogen source in place of ammonium sulphate.
Figure 4.2 Effect of glutathione and methionine as sulphur sources on the growth of met15Δ and met15Δ ecm38Δ strains. Strains ABC 733 (met15Δ) and ABC1083 (met15Δ ecm38Δ) grown in (A) Ammonium sulphate, (B) Glutamate. Open shapes, met15Δ; filled shapes, met15Δ ecm38Δ. □, No additions; ●, ○ GSH (250µM); ▲, △ methionine (250µM). Growth curve experiment was carried out essentially as described in Materials and Methods, section 2.2.7. Briefly cells were grown for 20 hrs in minimal SD media containing ammonium sulphate and methionine, washed twice with SD medium and reinoculated at an OD_{600} of about 0.1 in SD medium having ammonium or glutamate as nitrogen source and either methionine or glutathione as organic sulphur source. The growth was monitored by recording absorbance at 600 nm at definite intervals.
Fig 4.3 Growth of $met15\Delta$, $met15\Delta gsh1\Delta$ and $met15\Delta gsh1\Delta ecm38\Delta$ on SD plates having (A) methionine, (B) methionine+GSH, and (C) GSH as the sulphur source respectively. GSH and methionine were used at a concentration of 0.2mM.
Fig 4.4 Plate photograph showing the growth of met15Δ and met15Δecm38Δ on SD plates having γ-glutamylcysteine as the sole source of sulphur. γ-glutamylcysteine was used at a concentration of 0.2mM.
glutamylcysteine efficiently as an exogenous sole source of sulphur in the absence of γ-GT activity just like it was observed in case of GSH.

4.5 GSH utilization proceeds through the formation of cysteine since it requires a functional cysteine utilization pathway:

The studies described in the earlier section demonstrate that utilization of GSH, as a sole source of sulphur does not depend on the γ-GT activity of the yeast cells and proceeds through a γ-GT independent pathway. To get insights into the possible components of this alternative pathway we went on to check what are the intermediates being formed upon breakdown of GSH. We already know that S. cerevisiae can efficiently utilize organic sulphur sources like methionine, homocysteine, cystathionine, cysteine or GSH as a sole source of sulphur because of the presence of transulphuration pathways in which these compounds can rapidly exchange their sulphur atoms (Thomas and Surdin-Kerjan, 1997) (discussed in detail in Chapter 1: Introduction and Review). GSH degradation through this novel pathway might proceed through the generation of sulphate, homocysteine, cystathionine, methionine or cysteine or some other intermediate (Fig 4.5A). The most likely candidate appeared to be cysteine since it was a constituent amino acid of GSH. We examined this possibility by examining ABC1486 (str2Δ) strain of yeast. The STR2 gene encodes for Cystathionine γ-synthase, and str2Δ strains cannot utilize cysteine as the sole source of sulphur (Hansen and Johannesen, 2000). The str2Δ strain was checked for growth on plates having cysteine, GSH or methionine as a sole source of sulphur. The str2Δ did not grow when cysteine or GSH served as the sole source of sulphur but grew well on methionine or sulphate as the sole source of sulphur. The inability of str2Δ strain to grow on GSH suggests that GSH utilization requires a functional cysteine utilization pathway i.e. GSH degradation essentially proceeds by giving rise to cysteine (Fig 4.5B).

4.6 Glutathione disulphide (GSSG) can be utilized, as a sole source of exogenous sulphur in met15Δ strains independent of γ-GT and this ability is not affected in cells lacking glutathione reductase (Glr1p):

To investigate whether S. cerevisiae met15Δ cells in addition to utilizing reduced glutathione (GSH) could also utilize oxidized glutathione (GSSG) as a sole
Fig 4.5 (A) Possible routes of GSH utilization in *S. cerevisiae* through the alternative pathway; (B) Schematic representation of the sulphate assimilation pathway in *S. cerevisiae* showing that GSH utilization as a source of sulphur essentially proceeds through the formation of cysteine only. *MET15*: O-acetylhomoserine sulphhydrylase; *STR4*: Cystathione γ-synthase; *STR1*: Cystathionine γ-lyase; *STR3*: Cystathionine β-lyase; *STR2*: Cystathionine α-synthase. Tick mark (†) indicates the route taken by the alternate pathway.
source of sulphur. Growth experiments were carried out with GSSG as a sole sulphur source. The growth of met15Δ cells was found to be slightly slower on GSSG with a slightly longer lag phase as compared to growth on GSH (Fig 4.6). We also checked whether the utilization of GSSG is affected by the absence of γ-GT or glutathione reductase activity of the yeast cells. Therefore, we compared the growth of met15Δ, met15Δecm38Δ and met15Δglr1Δ on GSSG as the sole source of sulphur. No differences in growth or growth rates were observed between met15Δecm38Δ, met15Δglr1Δ and met15Δ cells (Fig 4.7). These observations clearly indicate that oxidized glutathione can also be utilized as a sole source of sulphur by S. cerevisiae met15Δ cells independent of the γ-GT and Glr1p activity.

4.7 Glutathione cannot be utilized as a sole source of nitrogen or as a source of glutamate in S. cerevisiae but can act as a source of glycine:

To examine, if in addition to the use of GSH as a sulphur source, yeast cells can grow using GSH as a nitrogen source, we examined the growth of wild type S. cerevisiae strain, ABC1338 (Y370-1D), on different sources of nitrogen. Wild type strain Y370-1D was chosen for this experiment as it is a prototrophic strain and hence does not require any amino acid supplements as growth requirement. (It was observed that supplementation of amino acids in the minimal medium even without any nitrogen source enabled the yeast cells to grow for few generations in case of strains that were auxotrophic for certain amino acids. This was possibly because the amino acids can act as a source of nitrogen for the cells). The growth of Y370-1D strain on 2mM GSH as source of nitrogen was similar to the growth of cells without any nitrogen source (Fig 4.8). Similar results were obtained with the use of 4mM or 6mM GSH as source of nitrogen. These results indicate that GSH cannot be utilized as a sole source of nitrogen by yeast cells unlike its efficient utilization as a sole sulphur source. The exact reason for this is unclear.

Further we went on to check whether GSH could be utilized as a source of glycine and glutamate by using mutant strains auxotrophic for either of these amino acids. GLY1 gene encodes for a threonine aldolase, a key enzyme in glycine biosynthesis and its deletion confers glycine auxotrophy when yeast cells are grown on glucose as the sole carbon source (Monschau et al., 1997). ACO1 gene encodes for a
Fig 4.6 Growth of ABC733 (met15Δ) cells on GSH (0.2mM) or GSSG (0.2mM) as the sole source of exogenous sulphur. Cells were initially grown overnight in SD medium containing methionine, washed twice with SD medium and then reinoculated in SD medium having either GSH or GSSG at an OD$_{600}$ of about 0.1. The growth was monitored by recording absorbance at 600 nm at regular intervals (described in detail in Materials and Methods, section 2.2.7).
Fig 4.7 Growth of \textit{met15}, \textit{met15:secm38} and \textit{met15:sglr1} on oxidized glutathione (GSSG) as the sole source of sulphur (GSSG: 0.2mM). Cells were grown overnight in SD medium containing GSH, washed twice with SD medium and reinoculated at an OD\textsubscript{600} of about 0.1 in SD medium containing GSSG as the sole source of sulphur. The growth was monitored by recording absorbance at 600 nm at regular intervals (described in detail in Materials and Methods, section 2.2.7).
Fig 4.8 Growth of wild type *S. cerevisiae* strain Y370-1D (ABC1338) on different sources of nitrogen. Cells were initially grown for 16 h in SD medium having Ammonium sulphate as the nitrogen source, washed twice with SD medium without any nitrogen source and reinoculated in SD medium containing individual nitrogen sources. Ammonium sulphate was used at a concentration of 5mg/ml while glutamate and proline were used at a concentration of 1mg/ml. GSH was used at a concentration of 2mM. The absorbance at 600 nm was recorded at regular intervals. (described in detail in Materials and Methods, section 2.2.7).
mitochondrial aconitase required for the tricarboxylic cycle and its deletion leads to glutamate auxotrophy.

We obtained gly1Δ and aco1Δ in a met15Δ strain background from EUROSCARF and checked both these strains separately on minimal media plates having GSH as a source of glycine or glutamate instead of glycine or glutamate respectively. We initially examined if GSH could act as a source of glutamate for the yeast cells. However no growth could be observed in case of met15Δ aco1Δ on plates having GSH as the sole source of glutamate and sulphur or on plates having both GSH + meth, while growth was observed on plates having glutamate along with GSH or methionine or cysteine (Fig 4.9). These results clearly suggest that GSH cannot be utilized as a source of glutamate by the yeast cells.

Subsequently we checked if GSH could act as a source of glycine and observed that met15Δ gly1Δ grew well just like met15Δ strain on plates having GSH, where it acts both as a source of glycine as well as sulphur (Fig 4.10). The met15Δ gly1Δ strain also grew well on plates having methionine + glycine or cysteine + glycine. In control plates having cysteine or methionine alone or on plates having glycine alone without any sulphur source no growth was seen as expected. One surprising observation was that no growth was observed on plates having both GSH + methionine, where methionine was added as a sulphur source and GSH as a source of glycine. The most likely explanation is that either the GSH utilization pathway is repressed or GSH transport.

**DISCUSSION:**

The results described in this chapter clearly demonstrate that the utilization of glutathione as a sulphur source in met15Δ strains is independent of the presence or absence of a functional γ-GT enzyme, and demonstrates for the first time that an alternative pathway for glutathione degradation and utilization - independent of γ-GT-exists in these cells. Prior to this report, the only enzyme known to be involved in the degradation of glutathione in all living cells has been γ-GT (Meister and Anderson, 1983).

The alternative γ-GT independent pathway for GSH degradation appears to differ quite distinctly from γ-GT pathway, in terms of its regulation. γ-GT was strongly
Fig 4.9 Growth of met15Δ and met15Δaco1Δ on SD plates having (A) glutamate+methionine, (B) GSH, (C) glutamate+GSH, and (D) GSH+methionine. In (A) and (C) glutamate was used at a concentration of 1mg/ml both as a nitrogen source as well as a source of glutamate. In (B) and (D) ammonium sulphate was used as a nitrogen source. GSH and methionine were used at a concentration of 0.2mM.
Fig 4.10 Growth of *met15Δ* and *met15Δgly1Δ* on SD plates having (A) glycine+methionine, (B) GSH, (C) methionine, (D) methionine+GSH, (E) glycine+GSH, (F) glycine+cysteine, (G) glycine, (H) cysteine. In (A), (E), (F) and (G) glycine was used at a concentration of 1mg/ml. GSH, methionine and cysteine were used at a concentration of 0.2mM.
regulated by the nature of the nitrogen source in the medium unlike the alternative pathway.

The alternative pathway for GSH utilization proceeds via cysteine since \textit{str2Δ} strain was unable to grow on GSH as a sole source of sulphur but grew on methionine as a sole source of sulphur.

Interestingly glutathione couldn't act as a sole source of nitrogen or glutamate whereas it could serve as a source of sulphur or cysteine for the yeast cells. Furthermore it could also act as a sole source of glycine for the cells. However GSH could not act as a source of glycine if methionine was present in the medium along with GSH. One possible reason for this result might be the strong repression of the glutathione transporter Hgt1p by methionine. An alternative explanation might be the strong repression of the GSH degradation pathway by methionine.