J. Cell Sci. **20**, 207–213 (1976)
Printed in Great Britain

DNA REPAIR SYNTHESIS IN HUMAN HETEROKARYONS

III. THE RAPID AND SLOW COMPLEMENTING VARIETIES OF XERODERMA PIGMENTOSUM

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SUMMARY

Patients with Xeroderma pigmentosum and defective DNA excision repair can be distinguished as a rapid (r-XP) and slow (s-XP) complementing variety. When fused with normal cells, fibroblasts from the r-XP are complemented rapidly and in the absence of protein synthesis while those from the s-XP are complemented slowly by a process partly, but not entirely, dependent on protein synthesis. Heterokaryons with different ratios of r-XP to s-XP nuclei (i.e. 1:1–5 and 1–5:1) and control heterokaryons containing one normal and 1–5 r- or s-XP nuclei show that if cell fusion and incubation is conducted in medium preventing protein synthesis, the r-XP cells do not complement the s-XP partner at all and, conversely, that the latter is not as effective as normal cells at complementing the r-XP partner. On the contrary, if protein synthesis is permitted, the 2 types of XP cells complement each other in a gene dose-dependent manner and to an extent similar to that observed in the control heterokaryons. These findings indicate that the r- and s-XP varieties are caused by mutations at different loci and suggest that the products of these loci interact to produce a functional unit which is present in normal control cells but absent in the XP strains. The relationship between the complementation groups described here and those already reported in the literature is being investigated.

INTRODUCTION

Xeroderma pigmentosum is an autosomal recessive condition characterized by hypersensitivity to sunlight followed by regressive changes and multiple recurring neoplasms in the exposed parts of the skin. Most of the patients with XP are defective in excision DNA repair while only few, called 'variants', have a defect in post-replication DNA repair (Lehmann *et al.* 1975).

Patients defective in excision repair are genetically heterogeneous and 5 complementation groups have so far been identified (Bootsma, De Weerd-Kastelein, Kleijer & Keijzer, 1975; Kraemer *et al.* 1975). Members of different complementation groups appear to be defective in the same step of the excision repair process (Paterson, Lohman & Sluyter, 1973; Cook, Friedberg & Cleaver, 1975) and the genetic and biochemical interpretation of the complementation findings is still somewhat uncertain.

Recently we have observed that while the fibroblasts from some XP patients are rapidly complemented by normal cells even in the absence of protein synthesis those

of others are complemented slowly by a process partly dependent on protein synthesis (Giannelli & Pawsey, 1974).

For simplicity's sake we have called rapid (r) and slow (s) these two types of XP, *r* and *s* the mutations they carry and *R* and *S* the respective wild type allele(s).

In order to understand better the functional and genetic relationship between the rapid and slow complementing type of XP, representatives of each class were crossed with each other and with normal controls. The kinetics of complementation were then studied quantitatively.

MATERIALS AND METHODS

The 4 fibroblast strains [male (H9172) and female (H9527) control; male s-type XP (H9500/PRU6947) and female r-type XP (H8199/PRU6221] were grown in Eagle's MEM with 10% foetal calf serum, penicillin and streptomycin. The XP fibroblasts were mixed in the 3:1 and 1:3 ratio with each other and in the 3:1 ratio with the normal control of opposite sex, fused with inactivated Sendai virus (Harris & Watkins, 1965) and plated in Petri dishes containing coverslips. One third of the virus-treated cells were incubated in medium containing 5 μg/ml of cycloheximide for 5 h in order to prevent protein synthesis, while the rest were incubated in normal medium for 5 and 23 h; 10 μ Ci/ml of [^{3}H]thymidine (sp. act. 25 Ci/mmol) were then added to the cultures in order to label differentially cells undergoing DNA replication. One hour later the cultures were washed in phosphate-buffered isotonic solution (PBS) irradiated with 100 J m^{-2} of ultraviolet light from a germicidal lamp emitting mainly at 254 nm and incubated for 1 h in fresh medium containing 10 μ Ci/ml of [^{3}H]thymidine (sp. act. 12·5 Ci/mmol) and, for the cultures where protein synthesis had been inhibited, 5 μg/ml of cycloheximide. The cells were then washed with PBS, fixed in 9:1 methanol:acetic acid and stained with Acranil: Chloromethoxyacridylaminodiethylaminopropanoldihydrochloride.

The following heterokaryons were selected for analysis and photographed: dikaryons to hexakaryons with 1 normal and 1–5 XP nuclei or 1 male s-XP and 1–5 female r-XP nuclei or, finally, 1 female r-XP and 1-5 male s-XP nuclei. The DNA repair synthesis of each nucleus was then measured by autoradiography.

RESULTS

Our results, summarized in Figs. 1–3, should be considered with regard to: (1) the behaviour of the 2 XP strains when fused with normal fibroblasts; (2) the pattern of complementation in the XP × XP crosses; (3) the temporal changes in complementation and (4) the comparison between the XP × XP and the 2 XP × normal crosses.

In keeping with our earlier observations (Giannelli & Pawsey, 1974; Giannelli, Croll & Lewin, 1973), the female r-XP strain shows a rapid improvement after fusion with normal partners in conditions where protein synthesis was prevented and also a tendency to a curvilinear regression of repair synthesis on gene dose. In this cross, normal and XP nuclei come rapidly to equilibrium and have therefore been represented by a single symbol and line (Fig. 1, line *a*). On the contrary, in similar conditions, the s-XP × normal cross shows a marked disequilibrium between the XP and normal nuclei because, after cytoplasmic fusion, the former achieve only a modest improvement while the latter maintain their normal level of repair (Fig. 1, lines *b*, *b'*). Upon incubation in medium permitting protein synthesis (Figs. 2, 3) the

regression relative to the cross between the control and the r-XP becomes steeper, but not significantly ($P > 0{\cdot}05$), while in the s-XP × normal cross the XP nuclei show a marked improvement (i.e. increase in elevation of line b') and a tendency to equilibriate with the normal control nuclei.

The XP × XP cross shows dramatic asymmetry of complementation. In the absence of protein synthesis the r-XP strain is complemented by the other to a degree directly proportional to the R gene dose in the heterokaryons while the s-XP strain is not

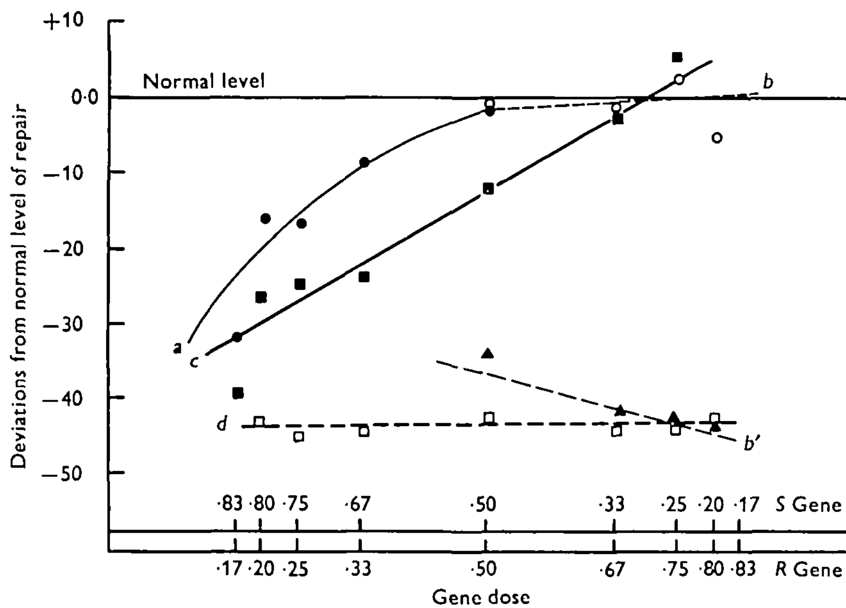

Fig. 1. Regression of DNA repair synthesis on gene dose in cycloheximide-treated heterokaryons. The levels of DNA repair synthesis of normal monokaryons in the XP × control crosses has been taken as the norm. This has been adjusted to 60 autoradiographic grains/nucleus and the DNA repair of nuclei in heterokaryons has been standardized accordingly. These standardized values have been expressed as deviations from the norm. The abscissa shows the dose of R or S genes in the heterokaryons. In the r-XP × normal cross, normal and XP nuclei always show similar grain counts and have therefore been represented by the same symbol (●). Line a (●—●) is the regression, on R gene dose, contributed by the latter cross. It departs significantly from linearity ($P < 0{\cdot}05$) and the data are better fitted by a quadratic regression ($P > 0{\cdot}05$). Line b (○---○) is the regression of normal nuclei in heterokaryons from the s-XP × normal cross on the S gene dose. The slope of this line does not differ significantly from zero ($P > 0{\cdot}6$) and runs very close to the normal level of DNA repair synthesis. The XP component of the heterokaryons from the latter cross, on the contrary, contributes a regression on S gene dose [line b' (▲---▲)] which is significantly different from zero ($P < 0{\cdot}001$) and has lower elevation than b. The XP × XP heterokaryons contribute 2 lines: the regression of the DNA repair synthesis of the r-XP nuclei on the R gene dose [line c (■——■)] which does not depart from linearity ($P > 0{\cdot}1$) and has a significant slope ($P < 0{\cdot}001$); and the regression of the s-XP nuclei on the S gene dose [line d (□--- □)] whose slope does not differ from zero ($P > 0{\cdot}7$). The a, b, b', c and d lines are based on: 505, 206, 367, 650, and 650 observations respectively. Note that lines a and c differ clearly in elevation and lines b' and d in slope ($P < 0{\cdot}001$).

complemented at all (Fig. 1, lines *c* and *d* respectively). After a brief period of protein synthesis the pattern of complementation markedly changes. Complementation of the r-XP component improves in heterokaryons with an excess of r-XP nuclei (Fig. 2, line *c*) while the regression contributed by heterokaryons with an excess of s-XP nuclei (Fig. 2, line *c'*) shows a negative slope on *R* gene dose ($P < 0.01$). The s-XP nuclei show considerable improvement but in heterokaryons with an excess of r-XP nuclei they begin to show the adverse effects of the *r* mutation (Fig. 2,

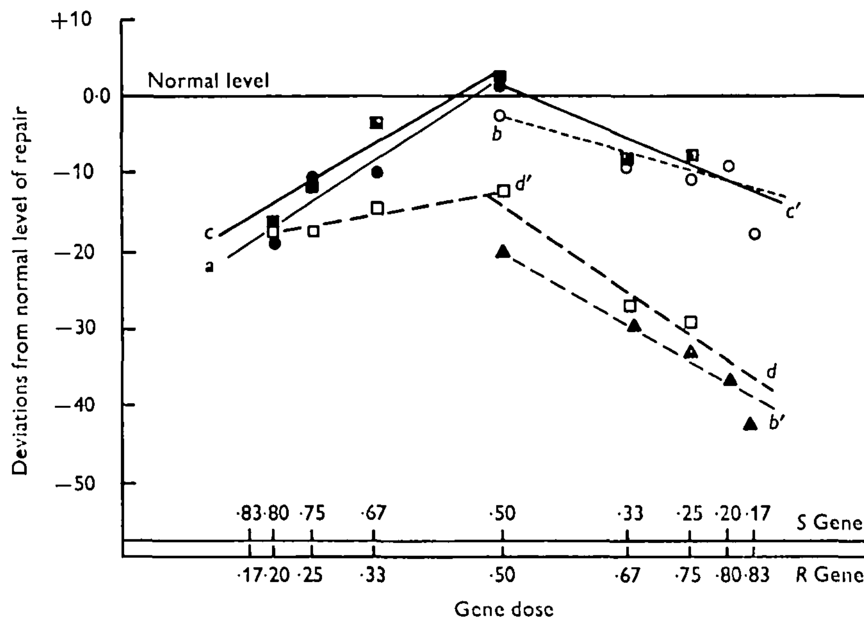

Fig. 2. Regression of DNA repair synthesis on gene dose in 6-h-old heterokaryons. Symbols and layout as in Fig. 1 except for lines *c* and *d* contributed by the XP × XP cross. The slope of these lines changes sign at the gene dose of 0·50 and the regressions with negative slope have been called *c'* and *d'*. These correspond to gene doses greater than 0·50.

None of the regressions shown in this figure depart significantly from linearity. The pairs of lines *a* and *c* and *b'* and *d* do not differ in slope ($P > 0.2$ and > 0.3 respectively) and only slightly in elevation ($P \simeq 0.05$ and < 0.01 respectively). Lines *c'* and *d'* show negative regressions ($P < 0.01$ and $P > 0.05$) on the dose of *R* and *S* genes, respectively, thus indicating that, in heterokaryons with an excess of s-XP nuclei (line *c'*), the *S* gene dose has an overriding effect while, in heterokaryons with an excess of r-XP nuclei (line *d'*), the *R* gene dose is of primary importance. The absolute values of the regression coefficients of lines *c'* and *b* do not differ significantly ($P > 0.6$) and the elevations of the 2 lines are similar. The lines *a*, *b*, *b'*, *c*, *c'*, *d*, *d'* are based on: 232, 108, 184, 467, 244, 393, and 287 observations respectively.

line *d'*). These changes become more obvious after 24-h incubation (Fig. 3) when cells with an excess of s-XP nuclei maintain the level of repair achieved by the r-XP nuclei during the first 6 h (Fig. 3, line *c'*) and continue to show improvement in repair synthesis of s-XP nuclei (Fig. 3, line *d*), at the same time, all the cells with an excess of r-XP nuclei show similar gene-dose-dependent negative effects of the *r* mutation (Fig. 3, lines *d'*, *c*).

Finally with regard to the comparison between the XP × XP and the 2 XP × normal crosses it is interesting to contrast the cycloheximide-treated heterokaryons with the others. The latter behave in a very similar fashion irrespective of whether they are from the XP × XP cross or those with controls, while the former behave differently in the different crosses. Namely, in the XP × XP crosses the r-XP nuclei are complemented to a degree clearly inferior to that in the control cross (Fig. 1: note different shape and elevation of lines *a* and *c*) and the s-XP nuclei are not complemented at all (Fig. 1, line *d*). The latter, in the control cross, show modest but significant improvement (Fig. 1, line *b*′ and Giannelli & Pawsey, 1974).

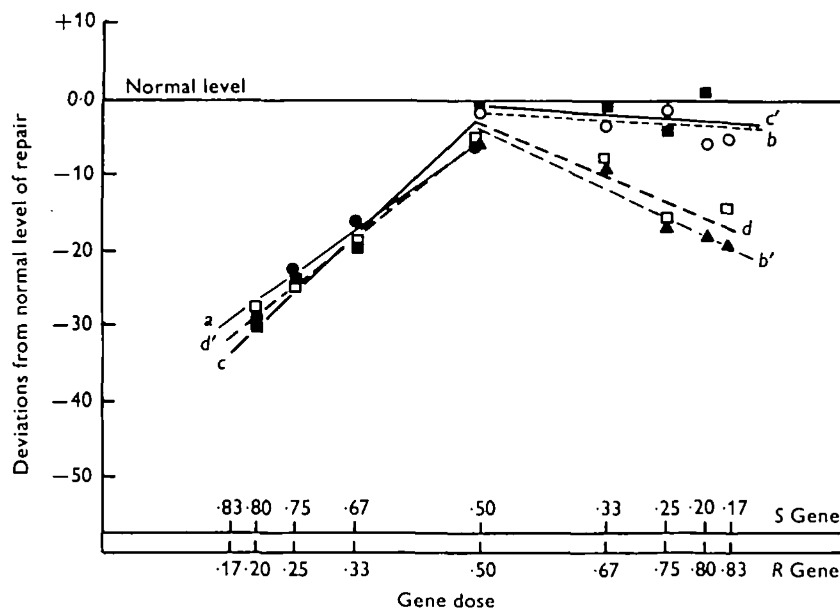

Fig. 3. Regression of DNA repair synthesis on gene dose in 24-h-old heterokaryons. Symbols and layout as in Fig. 2. Lines *a* and *c* show, as in Fig. 2, very similar slopes and elevations and so does line *d*′ whose negative regression on the *S* gene dose indicates an overriding effect of the *R* gene dose. Lines *c*′ and *b* nearly overlap each other and run very close to the normal level of repair. Lines *d* and *b*′, also, have similar elevations and similar slopes. The above lines in the order: *a*, *b*, *b*′, *c*, *c*′, *d*, *d*′ are based on 146, 101, 227, 417, 257, 397, and 240 observations.

N.B. Comparison of line *d*′ in Figs. 2 and 3 indicates a worsening, with time, of repair levels in heterokaryons with low *R* gene doses, while the elevations of lines *b*′ and *d* increase markedly from Fig. 2 to 3 ($P < 0.001$, $P < 0.001$) and indicate a clear time-dependent improvement of s-XP nuclei and a tendency to equilibrium between the different nuclei of heterokaryons with a low *S* gene dose.

DISCUSSION

The results summarized above suggest a number of conclusions, albeit tentative. For example, the cycloheximide-treated heterokaryons indicate that the products of the *R* and *S* genes interact: in the absence of interaction the XP × XP cross and those

with controls should show similar patterns of complementation both in the absence, and in the presence of protein synthesis.

The different kinetics and asymmetry of complementation in the XP × XP cross which has its counterpart in the behaviour of the XP × control crosses, and the observation that the gene-dose-dependent levels of DNA repair synthesis in the 24-h-old heterokaryons are not lower in the XP × XP than in the control crosses suggest that probably the *r* and *s* mutations are not allelic. This contrasts with a previously expressed view based on the observation that the heterozygous state for the *s* mutation could be detected using cycloheximide-treated heterokaryons with an excess of r-XP nuclei (Giannelli & Pawsey, 1974). Such findings, however, are consistent with the observation that cycloheximide-treated heterokaryons, with the same *R* gene dose, from the XP × XP and the r-XP × control cross, differ in their level of DNA repair synthesis (see Fig. 1, lines *a* and *c*) and may be explained by the interaction of non-allelic gene products.

Furthermore, the different kinetics of complementation of the r- and s-XP, and especially the different speeds with which the nuclei of these cells come to equilibrium with the nuclei of normal partners, indicate that the *S* gene product has a high affinity for the nucleus while the *R* gene product has not. We have suggested earlier that the apparently irreversible nuclear affinity of the *S* gene product might be due to the presence of a nuclear 'acceptor' (Giannelli & Pawsey, 1974).

The relationship between the *r* and *s* mutations and the 5 complementation groups already described in the literature is not yet clear. It is hoped that investigations in progress, while clarifying this point, will throw some light on the question of whether the complementation groups discovered so far represent mutations at 5 different loci or result from inter-and intra-genic interactions at a smaller number of loci.

We are grateful to Professor G. Belyavin for the gift of Sendai virus and to Drs J. C. Hay and E. J. Moynahan for the referral of the patients used in this study. Professor P. E. Polani has given us most valuable advice and constant support. We acknowledge the assistance of Mr L. Kelberman. This work was supported by the Spastics Society and the Cancer Research Campaign.

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(*Received* 28 *August* 1975)