

20. P. R. Gordon-Weeks and R. O. Lockerbie, *Neuroscience* **13**, 119 (1984).
21. C. Cotman, M. L. Blank, A. Moehl, F. Snyder, *Biochemistry* **8**, 4606 (1969).
22. V. J. Aloyo, H. Zweirs, W. H. Gispen, *J. Neurochem.* **41**, 649 (1983).
23. A. B. Oestreicher et al., *ibid.*, p. 331.
24. F. Katz, L. Ellis, K. H. Pfenninger, *J. Neurosci.* **5**, 1402 (1985).
25. P. N. E. deGraan et al., *Neurosci. Lett.* **61**, 235 (1985).
26. Y. Nishizuka, *Science* **225**, 1365 (1984).
27. W. L. Farrar, T. P. Thomas, W. B. Anderson, *Nature (London)* **315**, 235 (1985).
28. S. Ramón y Cajal, *Degeneration and Regeneration of the Nervous System* (Oxford Univ. Press, London, 1928), pp. 693–702.
29. C. Clemente, *Int. Rev. Neurobiol.* **6**, 257 (1964).
30. K. F. Meiri, K. H. Pfenninger, M. B. Willard, *Proc. Natl. Acad. Sci. U.S.A.* **83**, 3537 (1986).
31. O. H. Lowry, N. J. Rosebrough, A. L. Farr, R. J. Randall, *J. Biol. Chem.* **193**, 265 (1951).
32. R. K. Carlin, D. J. Grab, R. S. Cohen, P. Siekevitz, *J. Cell Biol.* **86**, 831 (1980).
33. L. A. Sternberger, *Immunocytochemistry* (Wiley, New York, 1979).
34. We thank S. Bock, I. Virag, and M. Siegel for technical assistance. Supported by NIH grants EY01117 and NS18103 to J.A.F., EY03718 to J.J.N., and NS20178 to J.H.P.S.; by the Isabelle Niemela Fund; and by a Searle Scholars Award to J.H.P.S. R.D.J. was supported by a National Research Service Award.

10 October 1985; accepted 30 May 1986

Chromosome Y-Specific DNA Is Transferred to the Short Arm of X Chromosome in Human XX Males

MEA ANDERSSON, DAVID C. PAGE, ALBERT DE LA CHAPELLE*

Y-chromosomal DNA is present in the genomes of most human XX males. In these cases, maleness is probably due to the presence of the Y-encoded testis-determining factor (TDF). By means of in situ hybridization of a probe (pDP105) detecting Y-specific DNA to metaphases from three XX males, it was demonstrated that the Y DNA is located on the tip of the short arm of an X chromosome. This finding supports the hypothesis that XX maleness is frequently the result of transfer of Y DNA, including TDF, to a paternally derived X chromosome.

IN MAMMALS, SEX IS DETERMINED BY the presence or absence of the Y chromosome; males have a Y chromosome while females do not. One or more genes on the Y chromosome induces the undifferentiated gonad to become a testis; subsequent steps in sex differentiation are the result of the action of hormones. The gene(s) responsible for testicular determination has been termed the "testis-determining factor" (TDF) (1). The biochemical nature of TDF

is unknown, but the gene has been localized to the short arm of the human Y chromosome (2–3).

Testes occasionally occur in the apparent absence of a Y chromosome. "XX males" are sterile men with small testes and an otherwise near-normal phenotype (4). Testicular differentiation in XX males might occur because of the presence of TDF in their genomes. To account for the acquisition of TDF and the anomalous inheritance of the

X-linked blood group Xg in many XX males, it has been suggested that an aberrant X-Y interchange occurred during meiosis in the fathers (5).

The results of recent studies support the X-Y interchange hypothesis. As determined with X-linked restriction-fragment length polymorphisms, most if not all XX males indeed have one maternally derived and one paternally derived X chromosome (6). Direct evidence of X-Y exchange was provided by an XX male who expressed his father's allele for 12E7, a Y-linked marker, but failed to express his father's allele for Xg (7). When the genomes of 19 XX males were tested for the presence of 23 Y-specific restriction fragments, 12 of the males were found to have one or more of these DNA segments, while seven had none (3, 8). The TDF gene is probably close to these anonymous Y-specific DNA sequences present in the majority of XX males. However, the chromosomal location of the Y-derived DNA in those XX males is not known. The in situ hybridization experiments reported here were designed to answer this question.

The hybridization probe we used is pDP105, which detects a Y chromosome-specific family of DNA sequences (9). By the study of XX males, XYp- females, and XYq- males, it has been determined that most sequences homologous to pDP105 are found on Yp (and are present in some XX males), while other homologous sequences are found on Yq (10). There is no hybridization to female DNA at moderate stringencies.

For this study, we selected three XX males (LGL105, LGL115, and WHT950) (11) whose genomic DNA hybridized with pDP105 in Southern blotting experiments. To substantiate the normal chromosomal localization of pDP105 sequences, we also studied two normal 46,XY males.

Probe pDP105 was hybridized to meta-

Table 1. Grain counts after in situ hybridization of probe pDP105 to metaphase chromosomes from two normal 46,XY males and three 46,XX males. For the normal males and XX male LGL115, the metaphases were obtained from phytohemagglutinin-stimulated 3-day cultures of whole blood. For XX males LGL105 and WHT950, the metaphases were from Epstein Barr virus-transformed lymphoblastoid cell cultures. The metaphases were spread on microscope slides and hybridized with nick-translated probe DNA according to standard techniques (16). The air-dried chromosome preparations were denatured in 70% formamide, 0.3M NaCl, and 0.03M sodium citrate for 2 minutes at 70°C and hybridized in 50% formamide, 0.3M NaCl, 0.03M sodium citrate, and 10% dextran sulfate for 12 hours at 40°C with the ³H-labeled probe (specific activity 9 × 10⁶ count/min per microgram of DNA) at a concentration of 30 to 40 ng/ml. After hybridization, the slides were rinsed in 50% formamide, 0.3M NaCl, and 0.03M sodium citrate at 39°C. The slides were coated with Kodak NTB emulsion, developed after 5 to 14 days of exposure and stained with 0.25% Wright's stain (Gurr, BDH Chemicals Ltd. Poole, England). The metaphases were photographed and the analysis of grain distribution was made on the photographic print. In an alternative method, the slides were first stained with quinacrine hydrochloride (17) and suitable cells photographed. The slides were then rinsed in water and stained with Wright's stain. The distribution of the grains was determined by marking the localization of the grains as seen in the microscope on the photomicrographs showing Q-banding. Clusters of grains were counted as one grain.

Individual	Number of mitoses	Number of grains			Number of cells with at least one grain	
		Total	On Y (%)	On Xp22 (%)	On Y (%)	On Xp22 (%)
Normal male 1	28	174	33 (19)	2 (1)	23 (82)	2 (7)
Normal male 2	28	200	36 (18)	2 (1)	20 (71)	2 (7)
XX male LGL105	31	164		14 (9)		13 (42)
XX male LGL115	28	110		24 (22)		19 (68)
XX male WHT950	52	224		37 (17)		34 (65)

M. Andersson and A. de la Chapelle, Department of Medical Genetics, University of Helsinki, 00290 Helsinki, Finland.

D. C. Page, The Whitehead Institute for Biomedical Research, Cambridge, MA 02142.

*To whom correspondence should be addressed.

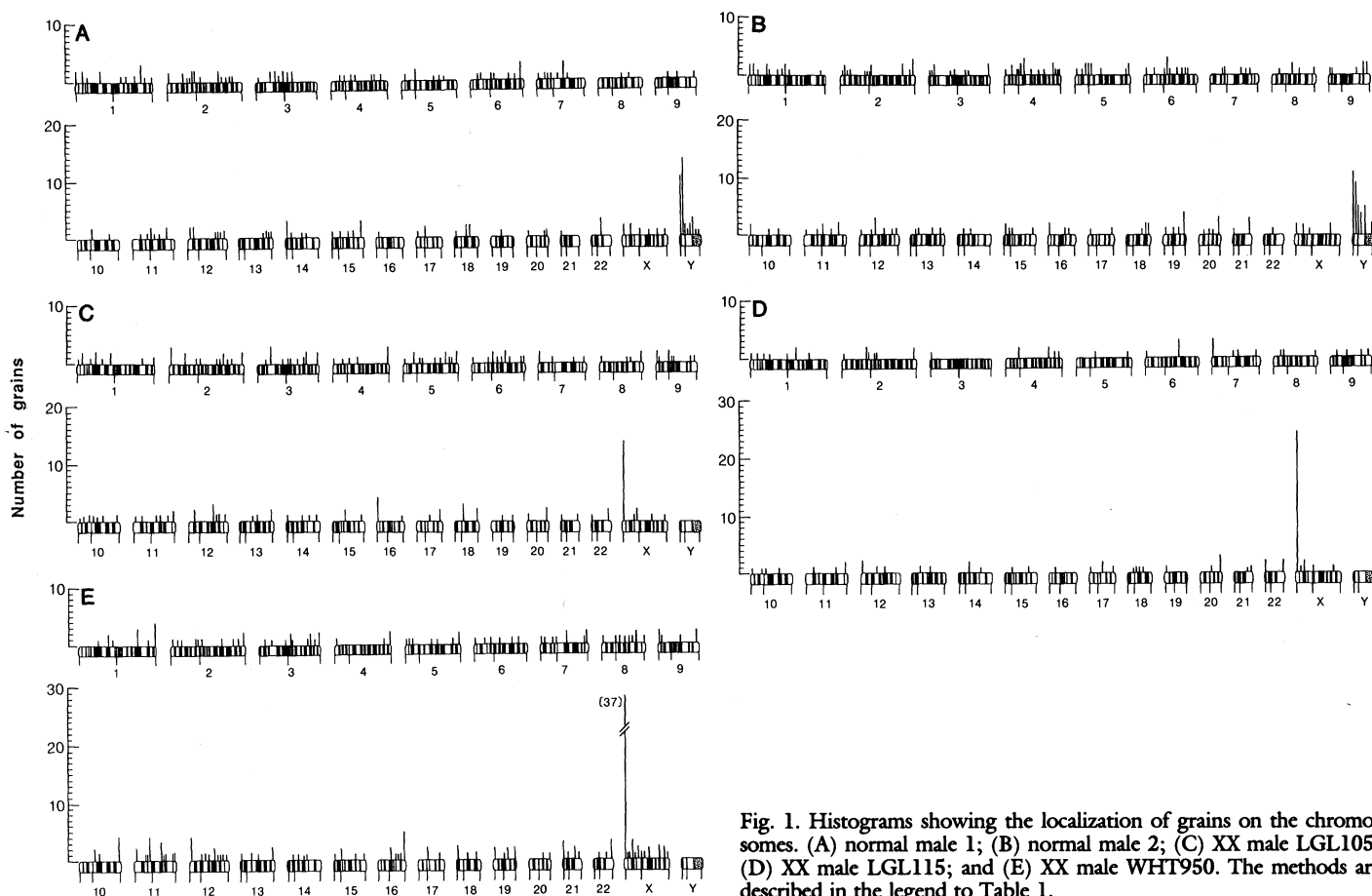


Fig. 1. Histograms showing the localization of grains on the chromosomes. (A) normal male 1; (B) normal male 2; (C) XX male LGL105; (D) XX male LGL115; and (E) XX male WHT950. The methods are described in the legend to Table 1.

phase chromosomes in situ (legend to Table 1). In the two normal 46,XY males, the Y chromosome was heavily labeled, while no other chromosome showed significant labeling (Fig. 1). Grains on the Y chromosome usually occurred in clusters of two to ten grains (median 3) while most other grains seen on the metaphase spreads were single. The distributions of grains on the chromosomes of males 1 and 2 are shown in Fig. 1. Consistent with the results of Southern blotting of genomic DNA's from cytogenetically abnormal individuals (10), we detected a major localization in Yp and a minor localization in Yq11.

In the three 46,XX males, grains were clustered on the distal portion of Xp (the short arm), with 9, 22, and 17% of the total chromosomal grains on Xp22 (Table 1). In no XX male mitosis did we find grains on the terminal short arm of both X chromosomes.

The distribution of grains on the chromosomes of XX males is shown in Fig. 1. The greatest concentration of grains occurred very distally on Xp, with a single peak on the terminal band Xp22 (see also Fig. 2). There was no significant labeling of autosomes. These findings indicate that Y-specific sequences detected by probe pDP105 have been transferred to distal Xp in these three

XX males. Our results exclude other chromosomal localizations in these XX males but do not, of course, exclude other localizations in other XX males. This finding lends support to the hypothesis that XX maleness is frequently the result of transfer of DNA from the Y to the X at male meiosis.

It has recently been demonstrated that the distal short arms of the human X and Y chromosomes regularly undergo recombination in male meiosis (12). While TDF does not lie within this pseudoautosomal region, it is located on Yp (3) and so may be close to the pseudoautosomal region. We have demonstrated that a TDF-bearing portion of Yp is transferred to Xp during or prior to meiosis in the fathers of XX males. This conclusion is in part based on the facts that in LGL105 and LGL115 one X is of paternal origin as shown previously (6) and that the mothers of these patients do not carry pDP105 (9). This abnormal Yp-to-Xp transfer may be related to the normal occurrence of recombination between these regions (13). This could account for the relatively high incidence of XX maleness—1 in 20,000 males (4).

In conclusion, our findings indicate that, at least in some XX males, Y chromosome-specific sequences occur on the distal end of the short arm of one X chromosome. This

supports the hypothesis that XX maleness in these cases arises through X-Y interchange involving TDF (5, 13). Our findings are also consistent with the observation of a cytogenetic alteration of the short arm of one X chromosome in XX males (14). The etiology of maleness in the absence of an apparent Y chromosome in man is similar, but not identical to, the Y-X interchange mechanism in *Sxr* mice (15).



Fig. 2. Photomicrograph of a Wright-stained metaphase from XX male WHT950 after hybridization with probe pDP105 and autoradiography. Arrow indicates cluster of grains on the distal region of the short arm of one of the X chromosomes. The methods are described in the legend to Table 1.

REFERENCES AND NOTES

1. T. B. Shows, P. J. McAlpine, R. L. Miller, *Cytogenet. Cell Genet.* **37**, 340 (1984).
2. R. M. Davis, *J. Med. Genet.* **18**, 161 (1981); R. E. Magenis *et al.*, *J. Pediatr.* **105**, 916 (1984).
3. G. Vergnaud *et al.*, *Am. J. Hum. Genet.* **38**, 109 (1986).
4. A. de la Chapelle, *Hum. Genet.* **58**, 105 (1981).
5. M. A. Ferguson-Smith, *Lancet* **1966-II**, 475 (1966).
6. D. C. Page and A. de la Chapelle, *Am. J. Hum. Genet.* **36**, 565 (1984).
7. A. de la Chapelle, P. A. Tippet, G. Wetterstrand, D. Page, *Nature (London)* **307**, 170 (1984).
8. G. Guellaen *et al.*, *ibid.*, p. 172.
9. D. C. Page, unpublished results. Probe pDP105 is a 5.5-kb genomic Hind III fragment inserted into the Hind III site of pUC13. The 5.5-kb fragment was isolated from the Y-specific library constructed at the Biomedical Sciences Division, Lawrence Livermore National Laboratory, Livermore, CA 94550; under the auspices of the National Laboratory Gene Library Project that is sponsored by the U.S. Department of Energy. Probe pDP105 defines human locus *DYZ4* [H. F. Willard, M. H. Skolnick, P. L. Pearson, J.-L. Mandel, *Cytogenet. Cell Genet.* **40**, 360 (1985)].
10. D. C. Page, unpublished results of deletion mapping by DNA hybridization, employing the logic described (3).
11. Details have been described (3). We thank Joseph Hershey, University of Louisville, Kentucky, for blood samples from XX male WHT950.
12. M.-C. Simmler *et al.*, *Nature (London)* **317**, 692 (1985); H. J. Cooke, W. R. A. Brown, G. A. Rappold, *ibid.*, p. 687; V. Buckle, C. Mondello, S. Darling, I. W. Craig, P. N. Goodfellow, *ibid.*, p. 739; F. Rouyer *et al.*, *ibid.* **319**, 291 (1986); D. C. Page *et al.*, in preparation.
13. P. E. Polani, *Hum. Genet.* **60**, 207 (1982); P. S. Burgoyne, *ibid.* **61**, 85 (1982); P. S. Burgoyne, *Nature (London)* **319**, 258 (1986).
14. H. J. Evans *et al.*, *Hum. Genet.* **49**, 11 (1979); R. E. Magenis *et al.*, *ibid.* **62**, 271 (1982).
15. L. Singh and K. Jones, *Cell* **28**, 205 (1982); E. P. Evans, M. Burtenshaw, B. M. Cattanaach, *Nature (London)* **300**, 443 (1982); B. M. Cattanaach, E. P. Evans, M. Burtenshaw, J. Barlow, *ibid.*, p. 445.
16. M. E. Chandler and J. J. Yunis, *Cytogenet. Cell Genet.* **22**, 352 (1978); M. E. Harper and G. F. Saunders, *Chromosoma* **83**, 431 (1981).
17. T. Caspersson, L. Zech, C. Johansson, E. J. Modest, *Chromosoma* **30**, 215 (1970).
18. We thank M. Nyström-Lahti, M.A., for helpful advice. Part of this study was done at the Folkhälsan Institute of Genetics, Helsinki. Supported by grants from the Sigrid Jusélius Foundation, the Finska Läkaresällskapet, the Academy of Finland, and the National Institutes of Health.

5 May 1986; accepted 20 June 1986

Active Human-Yeast Chimeric Phosphoglycerate Kinases Engineered by Domain Interchange

MARIA T. MAS,* CHRISTINA Y. CHEN, RONALD A. HITZEMAN, ARTHUR D. RIGGS

Phosphoglycerate kinase (PGK) is a monomeric protein composed of two domains of approximately equal size, connected by a hinge. Substrate-induced conformational change results in the closure of the active site cleft, which is situated between these two domains. In a study of the relations between structure and function of this enzyme, two interspecies hybrids were constructed, each composed of one domain from the human enzyme and one domain from the yeast enzyme. Despite a 35% difference in the amino acid composition between human and yeast PGK, catalytic properties of the hybrid enzymes are very similar to those of the parental proteins. This result demonstrates that the evolutionary substitutions within these two distantly related molecules do not significantly affect formation of the active site cleft, mechanism of domain closure, or enzyme activity itself.

IN MANY ENZYMES, DOMAINS (1, 2) CAN be viewed as functional units, involved in binding of substrates or cofactors. Binding of ligands frequently induces conformational changes within the domains, as well as relative domain movements (3). The importance of domain-domain interactions is particularly apparent for enzymes such as phosphoglycerate kinase (PGK), which have active sites located at the domain interfaces (2).

Phosphoglycerate kinases from yeast and horse muscle have very close structural homology (4–6) despite their 35% difference in amino acid sequence. Both proteins are composed of two globular domains of approximately equal size (22 kD), corresponding to the NH₂- and COOH-terminal halves of the molecule. Examination of a crystal structure of the yeast enzyme revealed a distance of about 10 Å between the inferred binding sites for the substrates, adenosine triphosphate (ATP) and 3-phosphoglycerate, each located on a different lobe of the enzyme (4, 5). It has been proposed that binding of substrates initiates a hinge-bend-

ing motion of the two domains, which results in the closure of an active site cleft (5, 7). A substrate-induced domain closure has also been postulated for other kinases (8). A crystallographic structure of phosphoglycerate kinase in the presence of both substrates is not available, and the nature of interactions involved in maintaining the closed conformation of the enzyme remains unknown. A precise arrangement of the amino acid residues on or near the interacting surfaces of both domains seems necessary for the formation of a functional catalytic center. Furthermore, many subtle intradomain and interdomain interactions may contribute to the mechanism of domain movement.

To study the importance of domain-domain interactions for (i) enzyme catalysis, (ii) protein folding, and (iii) protein stability, we have constructed two interspecies hybrids of phosphoglycerate kinase, each composed of a combination of one human and one yeast protein domain (Fig. 1). This approach exploits the 145 naturally occurring amino acid substitutions between the

species. Studying protein function by interchanging protein domains is a potentially powerful approach that exploits the natural variability of protein structure. Construction of hybrid α subunits of tryptophan synthetase, produced by in vivo recombination of *Escherichia coli* and *Salmonella typhimurium* genes, has been reported (9), but in vitro recombinant DNA techniques have not previously been used for this purpose. We report here that protein domains that have been evolutionarily separated for as much as 2 billion years (divergence of plants and animals) can still interact to yield enzymes with almost full activity.

A high-copy-number plasmid, YE9T (10), was used as a vector for the construction of the chimeric PGK genes—yeast-human PGK (yhPGK) and human-yeast PGK (hyPGK)—and for the expression of the chimeric and parental [yeast PGK (yPGK) and human PGK (hPGK)] genes (11). The yeast-human gene was constructed with the use of synthetic DNA (yeast sequence) to span a gap between the NH₂-terminal fragment of the yeast PGK gene, which had been cleaved at the Kpn I site, and the Nco I site in the human PGK coding sequence. The human-yeast hybrid gene was made with synthetic DNA (yeast sequence) to span a gap between the Nco I site (human PGK gene) and the Hpa II site in the yeast PGK gene. Therefore, these two constructions have the same junction point in the third base pair of the codon for a serine residue at position 172 in yeast PGK (174 in human PGK). This junction site in both hybrids is located within a highly conserved stretch of 16 amino acids (Fig. 2). The intact

M. T. Mas and A. D. Riggs, Division of Biology, Beckman Research Institute of the City of Hope, 1450 East Duarte Road, Duarte, CA 91010.
C. Y. Chen and R. A. Hitzeman, Department of Cell Genetics, Genentech, Inc., 460 Point San Bruno Boulevard, South San Francisco, CA 94080.

*To whom correspondence should be addressed.