can be checked by comparison with the spectrum of authentic human insulin (Fig. 2d). The spectra show both the B-chain and the molecular-ion region of the insulins, and the masses determined give the mass differences expected for the reactions on the assumption that the molecule is in its protonated form.

The sample amount used in the present study was ~10 μg of each protein. However, in the case of insulin, tests have indicated a distinct molecular-ion peak with as small an amount as 10 ng, that is, 2 pmol. The precision of the method is to some extent a function of the purity of the sample because an intense quasi-molecular ion peak gives a more accurate centroid determination. A high content of low-MW components seems to give a low signal-to-noise ratio. At present, the precision in the MW determination of components in the region up to MW 15,000 is better than 0.2 percent. This is approximately two orders of magnitude better than the precision of SDS-PAGE or high-performance gel permeation chromatography.

Our results demonstrate that PDMS is a viable method for MW determination up to 25,000. The precision obtained is sufficient to monitor even minor protein modifications. Therefore, possible applications are control of products from genetic engineering experiments and protein modification reactions in vivo and in vitro. We have also shown that the MW of components in a mixture can be determined and this for mixtures which are difficult or impossible to analyze with conventional techniques. The precision of the MW determination also makes PDMS useful for verifying sequence analysis.

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Human Ornithine Transcarbamylase Locus Mapped to Band Xp21.1 Near the Duchenne Muscular Dystrophy Locus

Abstract. *The gene for the mitochondrial enzyme ornithine transcarbamylase was mapped to the short arm of the X chromosome by in situ hybridization experiments, with DNA complementary to the human ornithine transcarbamylase gene used as a probe. A series of cell lines with X chromosome abnormalities was used to localize the gene to band Xp21.1. Because the gene maps near the Duchenne muscular dystrophy locus, the ornithine transcarbamylase probe may be useful in carrier detection and prenatal diagnosis of Duchenne muscular dystrophy as well as of ornithine transcarbamylase deficiency.*

Ornithine transcarbamylase (OTC), a trimeric enzyme composed of identical subunits, catalyzes the second step of the urea cycle. Although active in the mitochondrion, OTC is encoded by nuclear DNA and is X-linked in humans (*1*) and the mouse (*2*). Male infants, hemizygous for OTC deficiency, suffer from ammonia intoxication and protein intolerance; they often do not survive the

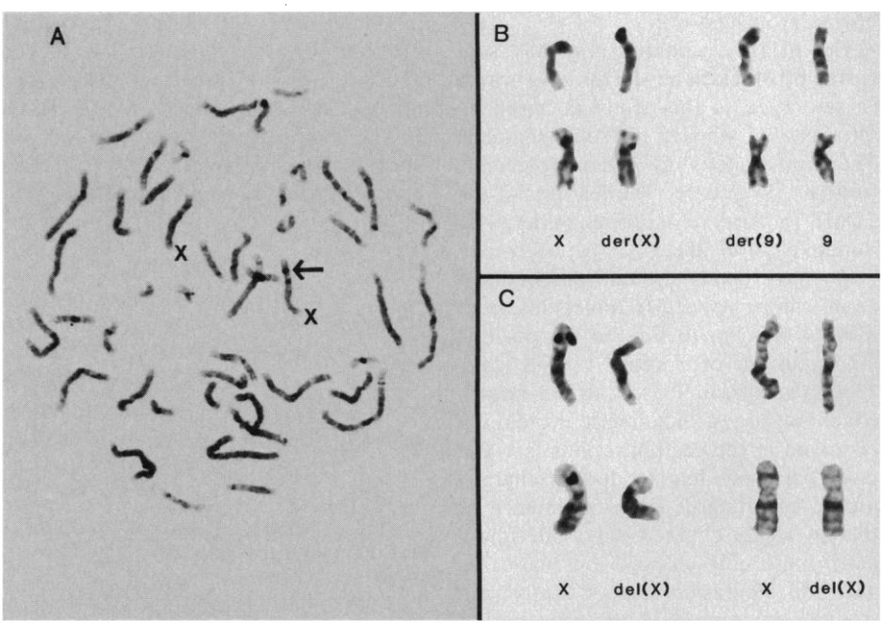

Fig. 1. Wright-stained chromosomes after in situ hybridization with plasmid pHO-731. Hybridizations followed the method of Harper and Saunders (*20*). The 1.5-kb Pst I fragment of OTC complementary DNA cloned into pBR332 was labeled with the tritiated triphosphates of deoxyadenosine, deoxycytosine, and thymidine, by nick translation to a specific activity 1.6×10^7 cpm/μg. The probe was hybridized to chromosome spreads overnight at a concentration of 25 ng/ml at 37°C. Photographic emulsion was applied to the slides, which were then exposed for 9 to 13 days. Chromosomes were stained with quinacrine mustard dihydrochloride (*21*) and photographed with a fluorescence microscope. The slides were then treated with Wright stain and, under bright light, a second photograph was taken of the cells previously chosen for analysis. (A) Representative normal human metaphase spread (46,XX) with a silver grain over the short arm of an X chromosome in the region of band p21 (arrow). (B) Partial karyotypes of two cells from lymphoblastoid cell line GM 6007 [46,X,t(X;9)(p21)(p22)], illustrating typical labeling of the der(X) and the X chromosomes in the region of band Xp21. (C) On the left, X chromosomes from two GM 7773 [46.X, del(X)(p21.1p21.3)] cells, showing labeling of the normal X short arm but not of the deleted X short arm. On the right, for comparison, are the X chromosomes from two GTG-banded cells that were not hybridized with the probe.

first week of life (*3*). Clinical manifestations in heterozygous females vary widely, as expected for an X chromosome locus undergoing random inactivation (*1*, *3*). Recently, a cloned probe for OTC, derived from a human liver complementary DNA library, has become available (*4*). We now report the results of in situ hybridization experiments in which we used tritiated plasmid pHO-731, containing a nearly full-length OTC cDNA, to assign the OTC gene to the short arm of the human X chromosome proximal to the Duchenne muscular dystrophy (DMD) locus.

The general location of the OTC gene on the X chromosome was identified by hybridizations to mitotic chromosome spreads containing structurally normal X chromosomes. These chromosome spreads were prepared using both lymphocytes from a normal female (Fig. 1A) and lymphoblasts from an established 48,XXXX cell line (GM 1416). Of 82 silver grains situated over X chromosomes, 63 (76.8 percent) were over bands Xp11.2 to p21, with a peak in the region Xp11.4 to Xp21.1 (Fig. 2A). No other chromosome region was labeled above background (*5*).

Since the region Xp11.2 to p21 comprises nearly half of the short arm of the X chromosome, additional hybridization experiments were carried out with cell lines containing structurally abnormal X chromosomes. The chromosome abnormalities had previously been demonstrated by high resolution banding studies. Lymphoblastoid line GM 6007 was established from a female with DMD and carries a balanced reciprocal translocation between the short arm of X and the short arm of 9 [46,X,t(X;9)(p21)(p22)] (*6*). This de novo chromosome abnormality putatively results in muscular dystrophy because the breakpoint in the abnormal X is at or near the DMD locus and the normal X is preferentially inactivated in somatic cells. After hybridization only the normal X and the der(X) translocation chromosome were labeled above background (Fig. 1B); the der(9) translocation chromosome, containing the distal region of the X short arm from the break in band p21 to the end of the arm, had only a background number of grains (Fig. 2B). Thus, the OTC gene is located closer to the centromere than is the DMD breakpoint in Xp21. Assuming that the DMD locus is at or very near the breakpoint, we propose the following gene order: centromere–OTC–DMD–telomere.

To define more precisely the location of the OTC gene, we hybridized the OTC probe with metaphases from a cell line that has material missing from the short arm of the X chromosome. Lymphoblastoid line GM 7773 is derived from a woman heterozygous for a de novo interstitial deletion of most of band Xp21 (*7*). All of these cells have a normal X chromosome as well as an X lacking bands p21.1 to p21.3 [46,X,del(X)(p21.1p21.3)]. In hybridizations with this cell line, the normal X displayed the expected labeling of the short arm (Fig. 1C). However, the deleted X was not labeled above background (Fig. 2C), indicating that the OTC gene had been deleted. Therefore, the sequence coding for OTC must be in band Xp21. This result, taken together with the other hybridization data, places the gene in band Xp21.1 (Fig. 3).

Somatic cell hybrids have been produced between rodent cells and cells containing the X chromosome abnormalities described above. Southern blotting of hybrid cell DNA with an OTC probe has independently generated results that are in complete agreement with the in situ data (not shown) (*8*).

In situ hybridization of labeled probes to chromosome preparations, combined with autoradiographic detection of ^{3}H or ^{125}I disintegrations, does not allow assignment of a DNA sequence to a single chromosome band. Because of the scatter of disintegrations in three dimensions through the photographic emulsion (*9*), grains are always observed over chromo-

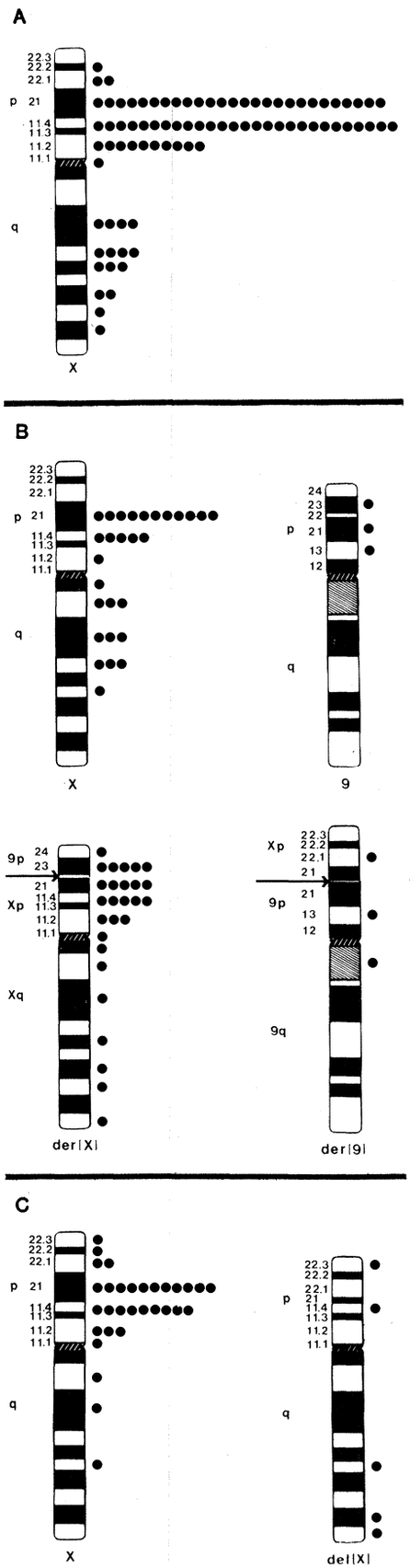

Fig. 2. Ideogrammatic representation of the distribution of silver grains after in situ hybridization with the OTC probe. Chromosomes were unambiguously identified on photographs of quinacrine-stained preparations, and then grains were scored at the microscope with the aid of bright-field photographs of the same cells. Although chromosome length varied between the 400- (metaphase) and the 850- (prophase) band stages, grains over chromosomes were conservatively scored on these 400-band-stage ideograms (*22*). (A) Distribution of grains over structurally normal X chromosomes from 46,XX cells and 48,XXXX cells (54 of each). Twenty-two of 214 grains (10.3 percent) in the 46,XX cells were over bands Xp11.2 to p21, and 41 of 199 grains (20.6 percent) in the 48,XXXX cells were over the same region. (B) Ideograms showing the grain distribution over the normal X and the normal 9 chromosomes, as well as over the reciprocal products of a translocation between chromosomes 9 and X, from GM 6007 [46,X,t(X;9)(p21)(p22)]. Arrows designate the breakpoints on the rearranged chromosomes. Seventeen (7.3 percent) of the total 234 grains scored in 66 cells were over bands Xp11.2 to Xp21; 18 (7.7 percent) were over the region Xp11.2 to 9p23 of the der(X). In contrast, only two grains (0.9 percent) were over the der(9) short arm, and three (1.3 percent) were over the short arm of the normal 9. (C) Grain distribution over the normal and deleted X chromosomes of GM 7773 [46,X,del(X)(p21.1p21.3)]. In 41 cells analyzed for grains over all chromosomes, 13 of 129 grains (10.1 percent) fell in the region Xp11.2 to p21 of the normal X, but only 1 (0.8 percent) fell in this (partially deleted) region of the abnormal X. The grains over the X chromosomes of 23 additional cells, scored only for X and deleted X, are included in this diagram.

some bands neighboring the actual site of the hybridizing sequence. Although the use of long prophase chromosome preparations mitigates this problem to some degree (*10*), the grains still scatter. It is also difficult to assign grains to specific bands in preparations that are marginally banded after hybridization, as is often the case. We have chosen an alternative approach toward increasing the precision of in situ hybridization by the use of cell lines with chromosome abnormalities that have been well defined by high resolution banding studies.

The precise localization of the OTC gene contributes significantly to the construction of a linkage map of the X chromosome made up of molecular markers. In one search for X-specific, single-copy sequences, only 3 of 16 clones (19 percent) were derived from the short arm of the X (*11*), although the short arm constitutes about 40 percent of the length of the X chromosome (*12*). The OTC sequence localization helps to fill in one of the remaining gaps.

A linkage map of the X chromosome will have many applications. Because approximately 120 X-linked loci have been identified in the human (*13*), every sequence precisely mapped on the X chromosome may be of use as a genetic marker for one or more X-linked disorders (*14*). Obviously, restriction fragment length polymorphisms (RFLP's) detected by the OTC probe would be of use in the prenatal diagnosis of OTC deficiency. Such polymorphisms have already been found (*15*). In addition, the OTC probe may be helpful in carrier detection and prenatal diagnosis of DMD, another very debilitating and much more common disorder. As reported here, the OTC gene maps closer to the DMD locus than do the anonymous RFLP loci identified by probe L1.28 [in Xp113-p11, estimated to be 17 centimorgans (cM) from the DMD locus] and probe RC8 (in Xp223-p21, also about 17 cM from the DMD locus), which have been suggested for use in prenatal diagnosis of DMD (*16*). A study of the linkage of polymorphisms at the OTC site with the DMD allele is in progress.

The localization of the OTC gene also sheds light on the evolutionary relationship between the X chromosomes of humans and mice. The X chromosomes of mammals are conserved so that, if a gene is mapped to the X in one species, it will be X-linked in others as well (*17*). However, the order of genes on the X may be different in different species (*18*). In keeping with this conservation of loci on the X, an analog of OTC deficiency called sparse fur (*spf*) has been mapped to the mouse X chromosome (*2*). In addition, a probable mouse analog (*mdx*) of human X-linked muscular dystrophy is localized on the mouse X chromosome (*19*). The order of these genes, as established by linkage analysis, is centromere–*spf*–*mdx*. This order is exactly the same as the order in humans. However, the mouse X is an acrocentric chromosome, whereas the human X is metacentric. These data suggest that the X chromosomes of the two species differ by a pericentric inversion, one breakpoint being distal to the DMD locus and the second at or just beyond the human X centromere in the long arm.

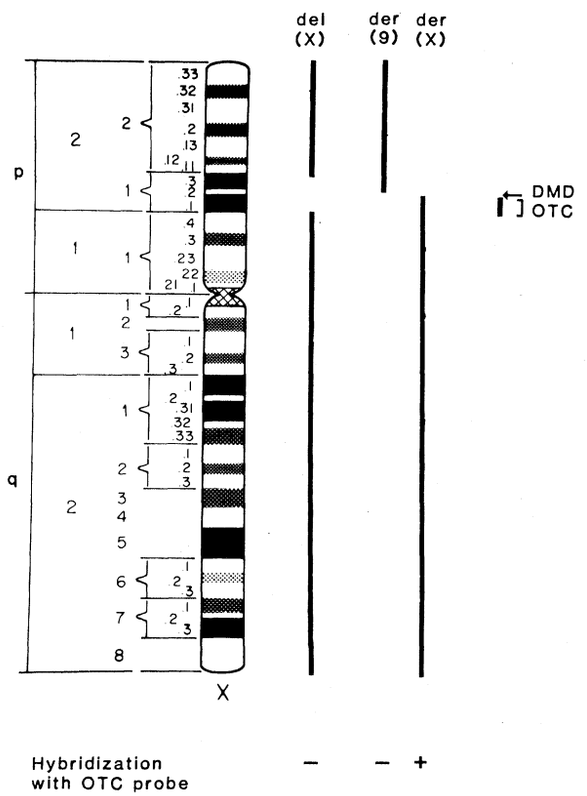

Fig. 3. Ideogram of the X chromosome at the 850-band stage (prophase), summarizing the in situ hybridization data obtained with the OTC probe. The deleted X of GM 7773 did not hybridize with the probe, nor did the der(9) chromosome of GM 6007. However, the der(X) chromosome of GM 6007 was labeled. The OTC sequence thus maps to band Xp21.1 and lies proximal to the DMD locus as defined by the breakpoint in GM 6007.

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