Mosaicism for Sulfoiduronate Sulfatase Deficiency in Carriers of Hunter's Syndrome

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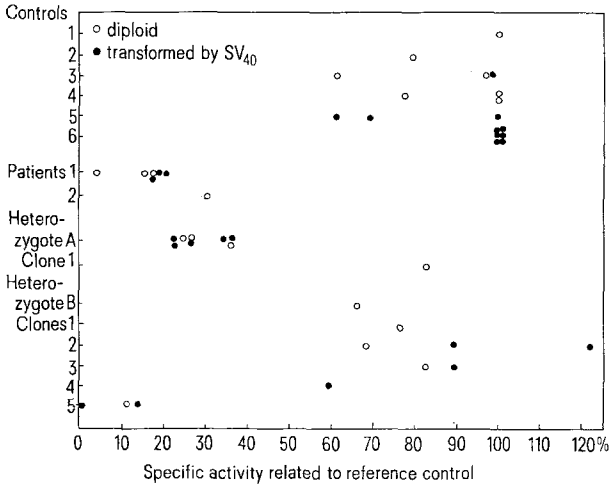
Summary. Using an assay for sulfoiduronate sulfatase based on the degradation of ^{35}S mucopolysaccharides in a cell-free system, two clonal populations have been demonstrated in fibroblasts of heterozygotes for Hunter's syndrome. The locus responsible for sulfoiduronate sulfatase deficiency in this *X*-linked mucopolysaccharidosis is therefore subjected to dosage compensation in females.

Hunter's syndrome (mucopolysaccharidosis II) is an *X*-linked storage disorder characterized by abnormal accumulation in affected males of dermatan sulfate and heparan sulfate[2], and by deficiency of sulfoiduronate sulfatase[3]. According to Lyon's hypothesis[4], female heterozygotes for *X*-linked markers are expected to show mosaicism in their somatic cells. Accordingly, 2 populations of clones have been observed in heterozygotes for glucose-6-phosphate dehydrogenase variants[5], hypoxanthine-guanine-phosphoribosyltransferase[6,7], α-galactosidase[8], phosphoglycerate kinase[9] and phosphorylase kinase[10] deficiencies. In addition, individual fibroblasts for Hunter's syndrome have been shown to be either negative or positive for metachromatic staining[11], a cellular phenotype common to different disease conditions[2]. However, the occurrence of metachromasia in fibroblast cultures is influenced by different environmental conditions, such as number of passage and growth phase of the cells[12], type of serum used for the culture[13] and alteration of pH of the medium[14]. The limitations in the use of metachromatic staining as a diagnostic tool are indicated by the high proportions of 'false-positive' tests: 27% in surveys of hospitalized patients taken at random[15] and 15% in fibroblast cultures initiated for different purposes[16].

It therefore appeared important, both from a genetic point of view and for its potential practical applications, to test whether the enzymatic defect of Hunter's syndrome is subject to lyonization, like the ones already mentioned. Using a quantitative enzymatic assay based on the degradation of ^{35}S-mucopolysaccharides, we have been able to detect 2 populations of clones in fibroblasts from 2 unrelated heterozygotes for this *X*-linked disorder, thus establishing a rational basis for the proper detection of carriers.



Specific activity of sulfoiduronate sulfatase in diploid fibroblasts (○). and fibroblasts transformed with SV-40 (●). Each point represents one assay of the SA of the individual or clone indicated on the ordinate with respect to a standard reference control taken as 100% SA, reported on the abscissa.

Fibroblast cultures and clones were obtained as previously described[8]. Radioactively labelled substrate was prepared by incubating fibroblasts from a patient affected with Hunter's syndrome with $MgSO_4$-free medium containing 0.1 mCi/ml of ^{35}S, according to published procedure[17]. In order to obtain optimal results, special care was given to the control of pH during the incorporation and chase of ^{35}S-mucopolysaccharides in the cultures[14].

Cell cultures to be assayed (one 100 mm Falcon dish) were trypsinized, washed in saline and broken by freeze and thawing (7 cycles) in 0.3 ml of 0.2 *M* sterile acetate buffer pH 4.1. The supernatant (75 µl) was incubated under sterile conditions with 25 µl of substrate (20.000 cpm) and 1 µl of 0.02% Na_3N for 70 h at 37 °C. The reaction was stopped by freeze-drying and the lyophilized material, resuspended in H_2O, was carefully spotted on Whatman No. 3 paper (16 × 20 cm) at 5.5 cm from the midline and electrophoresed in a Gelman chamber for 90 min at pH 8.6[18]. Free ^{35}S moved about 6 cm from the origin and the specific activity (SA) was calculated as cpm/mg protein/h. Protein concentration was measured using the Folin-Ciocalteau reagent[19]. In order to avoid corrections for the decay of ^{35}S in experiments peformed at different times, the same standard control fibroblast culture was run in each experiment and taken as 100% of SA.

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Uncloned and cloned cultures of fibroblasts were transformed with SV-40 as previously described[20] in order to obtain a vigorous cell growth, when needed. The phenotype of fibroblasts before and after transformation did not change in controls, patients and heterozygotes with respect to sulfoiduronate sulfatase activity (Figure).

Control fibroblasts showed a variable range of activity which was, however, well separated from that found in patients (Figure). It should be underlined that the residual activity found in affected males, corresponding to about 20% of the SA of the standard reference control, is probably due to ^{35}S containing substrates other than dermatan sulfate and heparan sulfate. The SA of uncloned fibroblasts from 2 heterozygotes fell in the range of affected males (heterozygotes A) or of controls (heterozygotes B). The single clone which was isolated from heterozygote A showed, however, a SA in the range of controls, while the majority of clones from heterozygotes B fell in the control range with only one (clone No. 5) showing an opposite phenotype. Both heterozygotes therefore showed some clones with sulfoiduronate sulfatase activity significantly different from that found in their uncloned cells and/or in the majority of their clones.

It has been reported that fibroblasts cultures from 5 obligate heterozygotes and 3 potential carriers of Hunter's syndrome displayed abnormal mucopolysaccharide metabolism after prolonged maintenance in culture or routine freezing[21]. Our data, in particular those from heterozygote B, apparently contradict the conclusion that there is preferential survival in culture of cells carrying the Hunter gene[21]. One should underline, however, that our experiments of cell cloning were performed at very early stages of subcultures. Since a reliable cell-free assay for the detection of Hunter's syndrome is now available, the specific basis for the reported abnormal mucopolysaccharide metabolism in cultures of heterozygotes can now be checked.

Another *X*-linked locus, that for the Xg^a blood group, whose linkage relationship with Hunter's locus is still undetermined, seems to escape inactivation also in view of recent findings which exclude the possibility that Xg^a substance is not produced by red cells and secondarily attached to their surface[22]. From a genetic point of view, it is therefore interesting that the mutation causing a deficiency of sulfoiduronate sulfatase in Hunter's syndrome is subjected instead to allelic inactivation. These apparently contrasting findings are in agreement with the hypothesis of regional inactivation of the same *X*-chromosome, supported also by recent data on derepression of the inactive *X*-chromosome at one single locus[23].

A new method for the assays of sulfoiduronate sulfatase based on the hydrolysis of a chemically prepared ^3H-labelled substrate is now available[24]. It should therefore become possible to refine the study of this newly discovered *X*-linked locus in future work concerning the mapping of the human *X*-chromosome[25].

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Rôle des phénomènes transitoires dans la reconnaissance spécifique du chant du Rouge-gorge (*Erithacus rubecula*)

The Role of Rise Time in the Recognition of Acoustic Elements in the European Robin's song (*Erithacus rubecula*)

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Summary. The rise time in the acoustic elements of the song is a very important parameter. The pattern of this rise is of little or no importance in recognition. The bird would appear to pay no attention to the fall time.

Un régime permanent de vibrations ne peut pas transmettre d'information. Pour atteindre ce but, on est contraint d'y apporter des modifications, c'est-à-dire de générer des phénomènes transitoires. Il en ressort que tout problème relatif à la transmission de l'information est étroitement lié à l'étude de ces phénomènes (PIMONOV[2]). Les signaux acoustiques des oiseaux comportent de nombreux phénomènes transitoires: ils consistent en des variations de fréquence associées à des variations de niveau. Nous nous proposons d'étudier le rôle des transitoires de niveau. En particulier nous chercherons à savoir si la forme exacte de la variation temporelle est un caractère important pour assurer l'efficacité des signaux.

Si de telles études sont souvent entreprises par les phonéticiens nous n'en connaissons qu'une seule qui soit relative au chant de l'oiseau (TRETZEL[3]). Il ne s'agit en fait que d'une tentative car l'auteur, comme il le reconnait lui-même, s'est heurté à des problèmes techniques que les moyens de l'époque ne permettaient pas de résoudre. Il eut toutefois le grand mérite d'être le premier à avoir pressenti et tenté de rechercher expérimentalement quelle était l'importance de ces transitoires pour l'oiseau récepteur.

Matériel. Les chants naturels. Ces derniers sont émis par des rouges-gorges sauvages. Les séquences ont été uniformément limitées à une durée d'une minute.

Enregistrement et diffusion. Le magnétophone utilisé est un Nagra III auquel est adjoint un amplificateur de puissance de 10 W et un haut-parleur pour la diffusion des signaux expérimentaux.

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