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Genetics

Demonstration, by somatic cell genetics, of coordinate regulation of genes for two enzymes of purine synthesis assigned to human chromosome 21

(mammalian genetic regulation/gene and protein organization)

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ABSTRACT **A method for determining coordinate genetic regulation is proposed for mammalian cells. The method involves (*i*) isolation of a set of mutants defective in the relevant pathway; (*ii*) complementation analysis of these mutants to determine dominance and to categorize the mutants into various different complementation groups; (*iii*) determination of the biochemical blocks in the mutants; (*iv*) identification of individual mutants that fail to complement the members of at least two distinct complementation groups that complement each other, such mutants being said to show coordinate regulation of the affected functions; (*v*) biochemical and reversion analysis of the relevant cell types to confirm the basis for the observed coordinate regulation; (*vi*) assignment of the individual genes to particular human chromosomes; (*vii*) mapping of the genes to determine contiguity on the genome; and (*viii*) examination of the structure of the relevant gene products. This method has allowed the demonstration of coordinate regulation between the gene coding for phosphoribosylglycineamide synthetase [5-phosphoribosylamine:glycine ligase (ADP-forming), EC 6.3.4.13], defective in our Ade$^-$C mutants, and the gene coding for phosphoribosylaminoimidazole synthetase [5′-phosphoribosylformylglycinamidine cyclo-ligase (ADP-forming), EC 6.3.3.1], defective in our Ade$^-$G mutants. Moreover, both genes can be assigned to human chromosome 21. Because at least two genes for purine biosynthesis have now been assigned to chromosome 21, and because patients with trisomy 21 (Down syndrome) show increased levels of serum purines, it may be that cells of these patients overproduce purines and that this overproduction may be relevant to the pathology of the syndrome.**

The organization of the genes coding for the enzymes of metabolic pathways in mammals is of fundamental interest. Such organization is certain to be relevant to regulation of gene function, yet little is known about this organization for enzymes in mammals. In certain cases, most notably the first three enzyme activities of pyrimidine biosynthesis, it has been possible to increase the levels of these three activities coordinately by gene amplification and to decrease these activities coordinately by mutation (1–3). Although the detailed organization and structure of the DNA coding for these activities remain unclear, it is highly likely that the coding regions will be relatively close to each other because the three activities reside upon a single polypeptide (1, 2). It would appear that a similar situation applies to the last two enzymes of UMP synthesis (4–7). No mammalian cell mutants with noncoordinate loss of one or another of the enzyme activities carried on a multifunctional protein with concomitant retention of the other activities has been reported.

The information to be gained from such studies should not be limited to those cases in which a multifunctional protein exists, because these cases may not reveal information regarding coordinate regulation of genes whose products are expressed as different polypeptides, regardless of the location of these genes. It should be possible to use a more general somatic cell genetic approach to look for genes that are coordinately regulated regardless of the organization of their gene products.

The general method that we propose involves several steps, all of which have been applied in one somatic-cell genetic system or another. These steps include (*i*) isolation of a set of mutants defective in the system of interest; (*ii*) complementation analysis of these mutants to determine dominance or recessiveness and to categorize the mutants into complementation groups; (*iii*) determination of the biochemical defects in the various mutants; (*iv*) identification of mutants that fail to complement members of at least two different complementation groups that complement each other, such mutants defining steps that are coordinately regulated; (*v*) reversion analysis of mutants showing coordinate regulation to demonstrate that these are not the result of large deletions but behave like point mutations; (*vi*) assignment of the involved genes to particular human chromosomes; (*vii*) mapping of any of these genes on the same chromosome to determine contiguity on the DNA, and isolation and sequence determination of the DNA in critical regions; and (*viii*) analysis of the structure of the involved gene products to determine whether multifunctional polypeptides or multienzyme complexes are involved. When such data become available, it should be possible to infer the molecular mechanism responsible for the observed coordinate regulation.

This method requires the existence of a number of genetically and biochemically well-characterized mutants in the enzymatic steps to be studied and is limited to those systems in which such mutants exist or can be readily generated. However, the method is not limited to nutritional mutants and has been partially applied to the study of cell-surface antigens (8). Of course, it also requires that appropriate somatic-cell hybrids can be prepared. For each set of mutants employed, coordinate regulation may or may not be found and negative results may reflect either lack of coordinate regulation or lack of appropriate mutants.

We chose to begin a search for coordinate regulation by examining members of the Ade$^-$C and Ade$^-$G complementation groups (Fig. 1) because several of the required steps of the method had already been achieved in this system. First, these have both been well-characterized biochemically (9, 10). Second, coordinate regulation of these genes has been found for the corresponding genes in *Schizosaccharomyces pombe* (11). Third, we possessed a number of mutants of each complementation group. Fourth, the *Ade$^-$C* gene in humans has been assigned to chromosome 21 (12). Study of genes on this particular

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Abbreviations: GARS, 5-phosphoribosylamine:glycine ligase (ADP-forming), EC 6.3.4.13; AIRS, 5′-phosphoribosylformylglycinamidine cyclo-ligase (ADP-forming), EC 6.3.3.1.

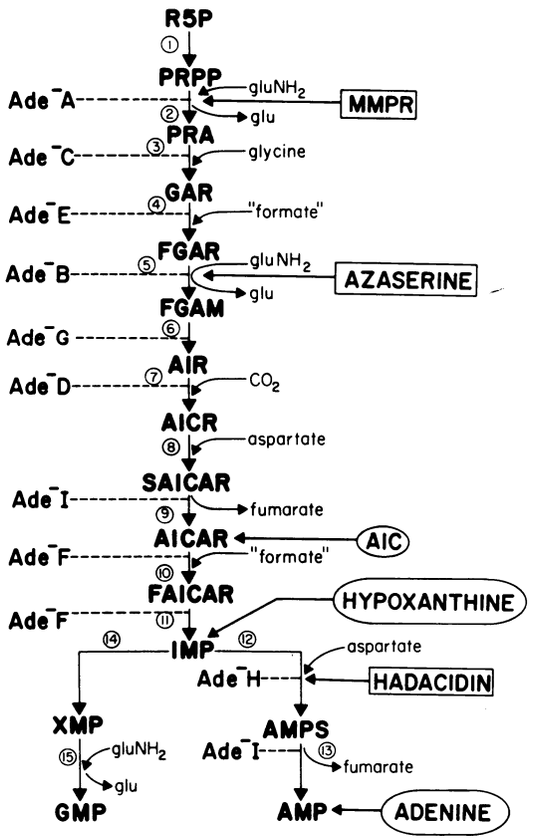

FIG. 1. A simplified representation of purine nucleotide biosynthesis. Details of the pathway may be found in refs. 23 and 24. MMPR, methylmercaptopurine riboside. Inhibitors of various steps are in squares, whereas useable purine intermediates are in ovals. Sites of defects in the various Ade⁻ mutant complementation groups are as shown.

human chromosome is, we think, of particular importance because trisomy 21, or Down syndrome, is the most common of the human aneuploid diseases. Moreover, the region of this chromosome, which needs to be trisomic to cause the syndrome, is quite small (as determined by the study of available translocations leading to Down syndrome) and may only carry a small number of genes (13–15). Therefore, if genes can be excluded from this region of the chromosome, they must not be involved in the pathology of the disease. On the other hand, if they are in this region of the chromosome, the chances are relatively high that they do play a role in the pathology of Down syndrome. Localization of genes known to be on chromosome 21 should be relatively straightforward by somatic cell genetic techniques. Thus, it may be that, among the aneuploidies, Down syndrome will be particularly amenable to attack with a somatic cell genetic approach.

MATERIALS AND METHODS

Cells and Media. The Chinese hamster ovary cell CHO-K1 (pro⁻) was the parental cell for all mutants used here. The Ade⁻C and Ade⁻G mutants have been characterized (9, 10). Cells were routinely grown in F12 medium supplemented with 8% (vol/vol) fetal calf serum as described (16) and with additional nutrients when required to meet additional auxotrophies.

Complementation Analysis. Complementation analysis of pairs was carried out as described (17).

Preparation of Human–Hamster Hybrids. Fusions between the Ade⁻G clone 55-1, which is LDH⁻ (18), and human lymphocytes were performed as described (12). Hybrid clones were isolated after growth in selective F12D medium.

Isozyme Analysis. The following 23 isozymes and 1 nutritional marker were assayed in the hybrid clones by using methods as described (19). Numbers in brackets correspond to human chromosome assignments (20): 6-phosphogluconate dehydrogenase [1]; malate dehydrogenase, soluble [2]; β-galactosidase [3]; phosphoglucomutase 2 [4]; arylsulfatase B [5]; superoxide dismutase, mitochondrial [6]; β-glucuronidase [7]; glutathione reductase [8]; adenylate kinase [9]; proline requirement [10]; lactate dehydrogenase A [11]; lactate dehydrogenase B [12]; esterase D [13]; nucleoside phosphorylase [14]; hexosaminidase-A and pyruvate kinase [15]; phosphogluconate phosphatase [16]; galactokinase [17]; peptidase A [18]; glucose phosphate isomerase [19]; adenosine deaminase [20]; superoxide dismutase, soluble [21]; arylsulfatase-A [22]; and glucose-6-phosphate dehydrogenase [X].

Karyological Analysis. The method for trypsin banding of metaphase chromosomes was described (21).

Biochemical Analysis. Cells were harvested with trypsin in the exponential phase of growth. After washing two times in saline D (12) they were frozen, thawed, and resuspended at 5–10 $\times 10^7$ cells per ml in saline D. After three freeze thaw cycles in a dry ice/acetone slurry, membranes were removed by centrifugation for 5 min in an Eppendorf centrifuge. The supernatant was collected and centrifuged at 100,000 $\times$ *g* for 60 min. The cell extract was then dialyzed overnight in 100 vol of saline D, which was changed twice. Protein concentration was determined by the method of Lowry *et al.* (22) by using bovine serum albumin as a standard, and the extracts were diluted with saline D to 2.5 mg of protein per ml. For all enzyme assays, specific activities were determined from a plot of activity vs. μg of protein in the linear part of the curve.

Phosphoribosylglycinamide synthetase [GARS 5-phosphoribosylamine:glycine ligase (ADP-forming), EC 6.3.4.13] activity was assayed by a procedure (9) modified to separate the product, glycinamide ribonucleotide (GAR), from the radiolabeled substrate, [^{14}C]glycine, by chromatography on Dowex 50W (NH_4^+ form) rather than by filtration on DEAE-cellulose discs. A control containing no enzyme gave a background of 342 cpm whereas a reaction mixture with 25 μg of CHO-K1 protein gave 66,000 cpm. Data presented are representative of at least four determinations.

Phosphoribosylaminoimidazole synthetase [AIRS; 5′ phosphoribosylformylglycinamidine cyclo-ligase (ADP-forming), EC 6.3.3.1] activity was assayed according to Flaks and Lukens (23) as modified by Irwin *et al.* (10). A reaction mixture containing 28 μg of CHO-K1 protein had an $A_{500} = 0.065$, whereas a control with no enzyme had an $A_{500} = 0.012$. Data presented are representative of at least four determinations.

Inosinicase [IMP 1,2 hydrolase (decyclizing), EC 3.5.4.10] was assayed by the method of Flaks and Lukens (23). A control with no enzyme had an $A_{540} = 0.192$ whereas a reaction mix containing 18.5 μg of CHO-K1 protein had an $A_{540} = 0.073$. Data presented are representative of at least two determinations.

Phosphoribosylformylaminoimidazolecarboxamide was prepared according to the method of Lukens and Flaks (24).

RESULTS

Isolation of a Mutant Failing To Complement Either Ade⁻C or Ade⁻G Mutants. To search for mutants that would not complement either Ade⁻C or Ade⁻G mutants, we hybridized all our independently isolated mutants assigned to the Ade⁻C and Ade⁻G complementation groups with each other. There were four independent mutants assigned to the Ade⁻C group and

Table 1. Complementation analysis of pairs of independent Ade⁻G and Ade⁻C clones*

	Ade⁻C (162)	Ade⁻C (16)	Ade⁻C (471)	Ade⁻C (405)	Ade⁻G (104)	Ade⁻G (C2C)	Ade⁻G (55)	Ade⁻G (PY5)
Ade⁻C (162)	0							
Ade⁻C (16)	0	0						
Ade⁻C (471)	0	0	0					
Ade⁻C (405)	0	0	0	0				
Ade⁻G (104)	19	5	8	0	0			
Ade⁻G (C2C)	44	14	84	0	0	0		
Ade⁻G (55)	28	12	12	0	0	0	0	
Ade⁻G (PY5)	19	16	35	0	0	0	0	0

* Ade⁺ colonies per 10^4 cells plated.

four assigned to the Ade⁻G group (Table 1). All of these clones except one, clone 405, showed classical complementation behavior. That is, none of the clones assigned to the Ade⁻C group complemented the other Ade⁻C clones, but they all complemented each of the Ade⁻G clones and vice versa. Clone 405, which originally was classified as a member of the Ade⁻C complementation group, clearly did not complement any Ade⁻C clones or any Ade⁻G clones. Each of these clones was able to complement Ade⁻F, which was wild-type for the *Ade⁻C* and *Ade⁻G* loci (data not shown).

Clone 405 was then subjected to complementation with representative members of all 7 of our hypoxanthine-requiring mutant complementation groups. Clone 405 again failed to complement members of the Ade⁻C and Ade⁻G complementation groups (Table 2), which complemented each other. Clone 405 did complement members of the Ade⁻A, B, D, E, and F complementation groups. Therefore, clone 405 was not a dominant mutant. Finally, because each representative of each of the complementation groups other than 405 used for the experiment shown in Table 2 complemented every other complementation group, these clones appeared to be straightforward recessive mutants.

Analysis of Enzyme Activities in Ade⁻ Mutants and Revertants. Analysis of the enzyme activities found in selected clones and cell types was then undertaken. The standard Ade⁻C clone, which would be expected to lack GARS activity but to possess AIRS activity, in fact showed such behavior (Table 3). Also as expected, Ade⁻G cells possessed GARS activity but were completely lacking in AIRS activity. Clone 405, renamed Ade⁻P_{CG}, was essentially completely lacking in both activities.

Table 2. Complementation analysis of clone 405*

	405	Ade⁻A	Ade⁻B	Ade⁻C	Ade⁻D	Ade⁻E	Ade⁻F	Ade⁻G
405	0							
Ade⁻A	36	0						
Ade⁻B	37	45	0					
Ade⁻C	0	6	20	0				
Ade⁻D	32	68	29	14	0			
Ade⁻E	39	76	101	26	38	0		
Ade⁻F	37	42	42	20	45	31	0	
Ade⁻G	0	14	58	32	17	59	11	0

* Ade⁺ colonies per 10^4 cells plated.

Table 3. Specific activities of GARS, AIRS, and inosinicase in CHO-K1, purine-requiring mutants, revertants, hybrids, and normal human cells*

	GARS	AIRS	Inosinicase
CHO-K1	2.98	0.33	13.2
Boiled CHO-K1	<0.01	<0.03	0.9
Ade⁻C	<0.01	0.31	21.3
Ade⁻G	0.45	<0.03	9.8
Ade⁻P_{CG}	<0.01	<0.03	8.9
Ade⁻P_{CG}r10	0.54	0.05	22.2
21/C†	0.26	0.14	9.2
725-18‡	1.73	0.06	11.5
Human fibroblast	0.36	0.04	4.9

* nmol/min per mg.
† Human–hamster hybrid, Ade⁻C as hamster parent.
‡ Human–hamster hybrid, Ade⁻G as hamster parent.

Ade⁻P_{CG} was originally isolated after mutagenesis with ethyl methanesulfonate, which is thought to act primarily as a point mutagen. Therefore, we thought it unlikely that a deletion or multiple mutation was responsible for its phenotype. To gain further information on this point, a spontaneous revertant of this clone was isolated that no longer required purines for growth. When GARS and AIRS activities were measured in this cell, Ade⁻P_{CG}r10, both activities were partially restored. The restoration appeared to be approximately 15–20% of normal in each case (Table 3). The fact that only partial activity of similar extent for each enzyme was regained is consistent with the idea that the revertant results from a single genetic change that affects both enzyme activities but does not restore them to wild-type levels. Thus, Ade⁻P_{CG} behaves as a point mutation at a locus that coordinately regulates expression of both the *Ade⁻C* gene and the *Ade⁻G* gene.

Assignment of the Gene Identified by the Ade⁻G Mutation to Human Chromosome 21. The *Ade⁻C* locus is located on human chromosome 21 (12). In order to assign the *Ade⁻G* locus to a human chromosome, two independent cell fusions between Ade⁻G mutants and human lymphocytes from two different normal individuals were performed, from which 14 primary clones that did not show a growth requirement for purines were isolated. The seven primary hybrids from the first fusion were analyzed for synteny with at least one enzyme coded for by each human autosome and the X chromosome. Because the highest degree of concordance was with soluble superoxide dismutase (*SOD1*) as shown in Table 4, a large number of secondary clones were isolated from two of the SOD1-positive primary hybrids after growth in purine-containing, nonselective medium. In every case, concordant segregation of SOD1 and the ability to grow in purine-free medium was demonstrated. The seven primary clones from the second fusion were also assayed for SOD1 and other selected isozymes. These data (Table 5) showed 95% concordance of *Ade G* with human *SOD1*.

All of the clones isolated in the first fusion and the two discordant clones from the second fusion were also analyzed cytogenetically. The five clones from the first fusion that were positive for human SOD1 activity had a chromosome resembling human chromosome 21 in size and banding pattern. The discordant clones from both fusions were scrutinized for small or minute chromosomes that might be broken chromosomes 21. Clone 725-15 had a modal chromosome number of 19 and 20, the same as the Ade⁻G parent had, and contained no identifiable human chromosome. The possibility that it is a revertant has not been excluded. Clone 725-33 was difficult to analyze because of an increase in the number of fragments and very small chromosomes and because of an increase in total chromosome number.

Table 4. Isozyme analysis in seven Ade$^+$G primary hybrid clones*

Hybrid	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	X
725-6	−	−	+	−	−	−	−	−	+	−	−	+	−	+	−	−	−	−	−	−	+	+	−
752-15	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
725-18	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	+	−	−
725-26	+	+	+	−	+	−	+	+	+	−	−	+	−	−	+	−	−	−	−	+	+	−	−
725-32	−	−	−	−	+	−	−	−	+	−	−	−	−	+	−	−	−	+	−	−	+	−	−
725-33	−	−	−	−	−	−	−	−	−	−	−	+	+	−	−	−	−	−	−	−	−	−	−
725-40A	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−
Concordance (%)	14	14	29	0	29	0	14	14	43	0	0	43	14	43	14	0	0	14	0	14	71	14	0

* Presence (+) or absence (−) of human marker assigned to indicated chromosomes. The human marker(s) assayed for each chromosome is listed in *Materials and Methods*.

The two discordant clones from the second fusion had a modal chromosome number of 21 and 22. No intact 21 was identifiable in either clone, but small chromosomes that could be broken chromosomes 21 were found. An intact chromosome 7 was identified in the clone that had a modal chromosome number of 21.

We measured the activities of GARS and AIRS in human cells and in hamster–human hybrids constructed between Ade$^-$C cells or Ade$^-$G cells and human cells. In both types of hybrid, restoration of the missing activity was observed, although the levels of restoration were low and more closely resembled the enzyme levels found in human cells (Table 3). These results show that the enzyme activities missing in the Chinese hamster cell mutants were indeed restored in the Ade$^+$ cells isolated from the fusions and thus strongly argue against the possibility that these cells had activated an alternate pathway for meeting their purine requirements.

Thus, from the isozymic, biochemical, and cytogenetic data, we conclude that in humans the gene coding for AIRS, like the gene coding for GARS, is carried on human chromosome 21.

Also depicted in Table 3 is a measure of the activity of another enzyme of *de novo* purine synthesis in all these strains, inosinicase, which is substantial in all the cell types. Thus, the Ade$^-$P$_{CG}$ enzyme extract does indeed possess the activity of at least one of the *de novo* purine biosynthetic enzymes.

DISCUSSION AND CONCLUSIONS

In this work we describe a method for studying coordinate regulation of genes in mammalian cells. The method as currently formulated consists of eight steps, six of which have been applied to a system involving two genes of the purine biosynthetic pathway. The remaining two steps, which involve study of the structure of the relevant DNA segments and of the structure of the relevant protein gene products, should elucidate the nature of the coordinate regulation uncovered by using this method.

It can be concluded from this data that in Chinese hamster cells the genes for GARS and AIRS, the third and sixth enzymes of *de novo* purine biosynthesis (Fig. 1), are coordinately regulated and perhaps physically associated, because it is possible to eliminate both activities genetically and to restore both activities to approximately the same extent by reversion. Since Ade$^-$P$_{CG}$ does complement representative members of all of our other hypoxanthine-requiring complementation groups (Table 2), a mutation inactivating a diffusible regulatory molecule is unlikely. A mutation in a regulatory element on the same piece of DNA containing the structural genes for AIRS and GARS is consistent with the assignment of both genes to human chromosome 21 (9) (Tables 4 and 5) and with the coordinate restoration of both activities in the revertant Ade$^-$P$_{CG}$r10. Further study of mutants such as Ade$^-$P$_{CG}$ may reveal as yet unsuspected genetic regulatory mechanisms in the mammalian genome.

Table 5. Demonstration of synteny between *Ade G* (AIRS) and *SOD1*

	Number of clones *Ade G/SOD1*			
	+/+	+/−	−/+	−/−
Primary clones (fusion 1)	5	2	0	0
Primary clones (fusion 2)	5	2	0	0
Secondary clones of 725-6	12	0	0	24
Secondary clones of 725-18	4	0	0	19
Total	26	4	0	43

The physical basis for the coordinate regulation of these genes remains unknown. For example, Ade$^-$P$_{CG}$ could be a polar mutation such that a defect has occurred in the first of these two structural genes to be transcribed that effectively eliminates further transcription of either gene. Alternatively, the defect could be in the processing of the RNA transcript. Given the recent findings of transcribed noncoding and nontranscribed DNA segments within and between genes, it becomes difficult to draw conclusions regarding the physical structure and arrangement of genes from data similar to that presented here. Thus, for example, if Ade$^-$P$_{CG}$ is a polar mutant as described above, this is certainly not conclusive evidence that the DNA segments coding for the structural polypeptides need be contiguous on the DNA. It would appear that understanding of such physical arrangements would most easily be obtained by isolation and characterization of the relevant DNA segments and by mapping of the relevant DNA segments, as has been carried out for the globin genes by using a combined recombinant DNA/somatic-cell genetic approach (25).

In *Schizosaccharomyces pombe*, evidence similar to that presented here has been interpreted to mean that GARS and AIRS activities are present together on a multifunctional polypeptide (11). We have no definitive evidence as yet on this point in mammalian cells, although certain of the data presented in Table 3—namely, the reduced GARS activity in Ade$^-$G, the coordinate loss of both activities in Ade$^-$P$_{CG}$, and the coordinate restoration of activities of GARS and AIRS in the revertant—are consistent with such a hypothesis. It is clear that examples of multifunctional polypeptides are available in nucleotide metabolism in mammalian cells. The clearest example is the first three enzymatic steps of pyrimidine synthesis, which have been shown to be carried on a single polypeptide (1, 2).

Regardless of whether the GARS and AIRS activities are carried on the same or different polypeptides, the system described here behaves differently from any system thus far described in mammalian cells, because most of the independently isolated mutants (*i*) fall into one or the other complementation

group, (*ii*) lack one of the two activities, and (*iii*) complement all the members of the other complementation group. Mutants such as $Ade^{-}P_{CG}$ that lack both activities, that do not complement any of the mutants defective in either individual enzyme activity, and that behave in reversion studies as point mutations can be isolated, though these appear to be somewhat less common. Recently, we have found a similar but not identical situation involving members of our $Ade^{-}A$ and $Ade^{-}B$ complementation groups (26). Again, most of the mutants belong to the $Ade^{-}A$ or the $Ade^{-}B$ complementation groups, but mutants can be isolated that do not complement either. It would be of great importance to evaluate whether or not these enzyme activities are indeed on multifunctional polypeptides, are associated in a noncovalently linked enzyme complex, or are not functionally associated. In other reported cases of either coordinate loss or coordinate elevation of enzyme activities, which apparently involve multifunctional polypeptides, it has not yet proven possible, to our knowledge, to isolate single-step mutants that have noncoordinately altered the expression of the activities carried on these polypeptides. Because such phenomena seem so common in these metabolic pathways and because considerable conservation at least of these genetic and protein organizations during evolution has occurred, it is tempting to hypothesize that they must play some fundamental role in genetic-biochemical regulation of gene and enzyme activities in mammalian cells.

Appropriate regulation of purine and pyrimidine metabolism is in itself of crucial importance for normal human development, because abnormalities in these pathways do lead to developmental disorders, including orotic aciduria, Lesch-Nyhan syndrome, and at least two types of immune deficiency disease (27–30). In this context, it is significant that both GARS and AIRS map to human chromosome 21, the chromosome that when trisomic leads to Down syndrome, the most common human autosomal chromosome disease. It has been known for some time that Down syndrome patients possess elevated serum purine levels (31). Such elevations have been considered to be caused by decreased renal clearance of urate, although the evidence on this point is not convincing (32). GARS activity seems to be expressed according to gene dosage in human fibroblasts with different numbers of chromosome 21 (33, 34). In the light of these findings, the basis for elevated serum purines in these patients should be reinvestigated. If overproduction of purines is found in cells trisomic for chromosome 21, it will be necessary to determine whether this overproduction has any relevance to any of the pathology seen in Down syndrome. Regardless of whether a relationship between aberrant purine synthesis and Down syndrome is found, we consider it highly likely that the somatic-cell genetic method proposed and applied here will be widely applicable to the study of such human chromosomal diseases, which so far have not been elucidated by other methods, and will reveal significant information regarding the structure and regulation of the mammalian genome.

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1. Coleman, P. F., Suttle, D. P. & Stark, G. R. (1977) *J. Biol. Chem.* **252**, 6379–6385.
2. Davidson, J. N. & Patterson, D. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 1731–1735.
3. Padgett, R. A., Wahl, G. M., Coleman, R. F. & Stark, G. R. (1979) *J. Biol. Chem.* **254**, 974–980.
4. Suttle, D. P. & Stark, G. R. (1979) *J. Biol. Chem.* **254**, 4602–4607.
5. Patterson, D. (1980) *Somatic Cell Genet.* **6**, 101–114.
6. Krooth, R. S., Hsiao, W. L. & Potvin, B. W. (1979) *Somatic Cell Genet.* **5**, 551–570.
7. Levinson, B. B., Ullman, B. & Martin, D. W. (1979) *J. Biol. Chem.* **254**, 4396–4401.
8. Jones, C., Moore, E. E. & Lehman, D. W. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 6491–6495.
9. Oates, D. C. & Patterson, D. (1977) *Somatic Cell Genet.* **3**, 561–577.
10. Irwin, M., Oates, D. C. & Patterson, D. (1979) *Somatic Cell Genet.* **5**, 203–216.
11. Fluri, R., Coddington, A. & Flury, W. (1976) *Mol. Gen. Genet.* **147**, 271–282.
12. Moore, E. E., Jones, C., Kao, F. T. & Oates, D. C. (1977) *Am. J. Hum. Genet.* **29**, 389–396.
13. Hagemeijer, A. & Smit, E. M. E. (1977) *Hum. Genet.* **38**, 15–23.
14. Sinet, P. M., Couturier, J. Dutrillaux, B., Poissonnier, M., Raoul, O., Rethore, M. O., Allard, D., Lejeune, J. & Jerome, H. (1976) *Exp. Cell Res.* **97**, 47–55.
15. Kurnit, D. M. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 2372–2375.
16. Ham, R. G. (1965) *Proc. Natl. Acad. Sci. USA* **53**, 288–293.
17. Patterson, D., Kao, F. T. & Puck, T. (1974) *Proc. Natl. Acad. Sci. USA* **71**, 2057–2061.
18. Stamato, T. D. & Jones, C. (1977) *Somatic Cell Genet.* **3**, 637–647.
19. Jones, C., Kao, F. T. & Taylor, R. T. (1980) *Cytogenet. Cell Genet.*, in press.
20. Evans, H. J., Hamerton, J. L., Klinger, H. P. & McKusick, V. A., eds. (1979) *Birth Defects: Original Articles Series* (The National Foundation–March of Dimes, White Plain, NY), Vol. 15, No. 11.
21. Kao, F. T., Jones, C. & Puck, T. T. (1973) *Proc. Natl. Acad. Sci. USA* **73**, 193–197.
22. Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **19**, 265–275.
23. Flaks, J. G. & Lukens, L. (1963) *Methods Enzymol.* **6**, 52–95.
24. Lukens, L. & Flaks, J. G. (1963) *Methods Enzymol.* **6**, 671–702.
25. Gusella, J. F., Keys, C., Varsanyi-Breiner, A., Kao, F. T., Jones, C., Puck, T. T. & Housman, D. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 2829–2833.
26. Oates, D. C., Vannais, D. & Patterson, D. (1980) *Cell* **20**, 797–805.
27. Krooth, R. S. (1964) *Cold Spring Harbor Symp. Quant. Biol.* **29**, 189–212.
28. Lesch, M. & Nyhan, W. L. (1964) *Am. J. Med.* **36**, 561–569.
29. Giblett, E. K., Anderson, J., Cohen, P., Pollara, B. & Meuwissen, H. J. (1972) *Lancet* **ii**, 1067–1069.
30. Giblett, E. R., Ammann, A. J., Wara, D. W., Sandman, R. & Diamond, L. K. (1975) *Lancet* **i**, 1010–1013.
31. Fuller, R. W., Luce, M. W. & Merz, E. P. (1962) *Science* **137**, 868–869.
32. Watts, R. W. E., Perera, Y. S., Allsop, J., Newton, C., Platts-Mills, T. A. E. & Webster, A. D. B. (1979) *Clin. Exp. Immunol.* **36**, 355–363.
33. Scoggin, C. H., Bleskan, J., Davidson, J. N. & Patterson, D. (1980) *Clin. Res.* **28**, 31A.
34. Bartley, J. A. & Epstein, C. J. (1980) *Biochem. Biophys. Res. Commun.* **93**, 1286–1289.