Psych 3102
Introduction to Behavior Genetics

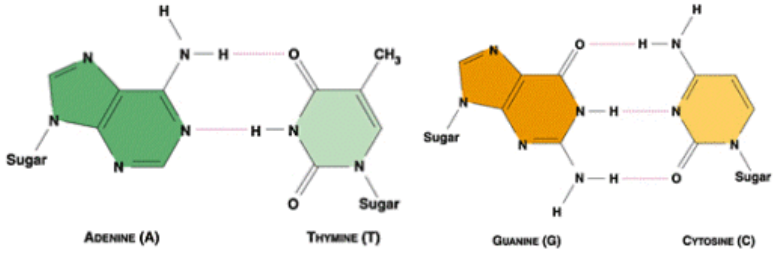
Lecture 6

Nature of the genetic material

Review: Structure and function of DNA

- Watson & Crick, 1953
- nucleic acid

  - chemical group to which RNA and DNA belong
- nucleotide
  - building block of nucleic acids
  - 3 subunits: 1.pentose sugar 2. phosphate group
    3. nitrogen-containing base

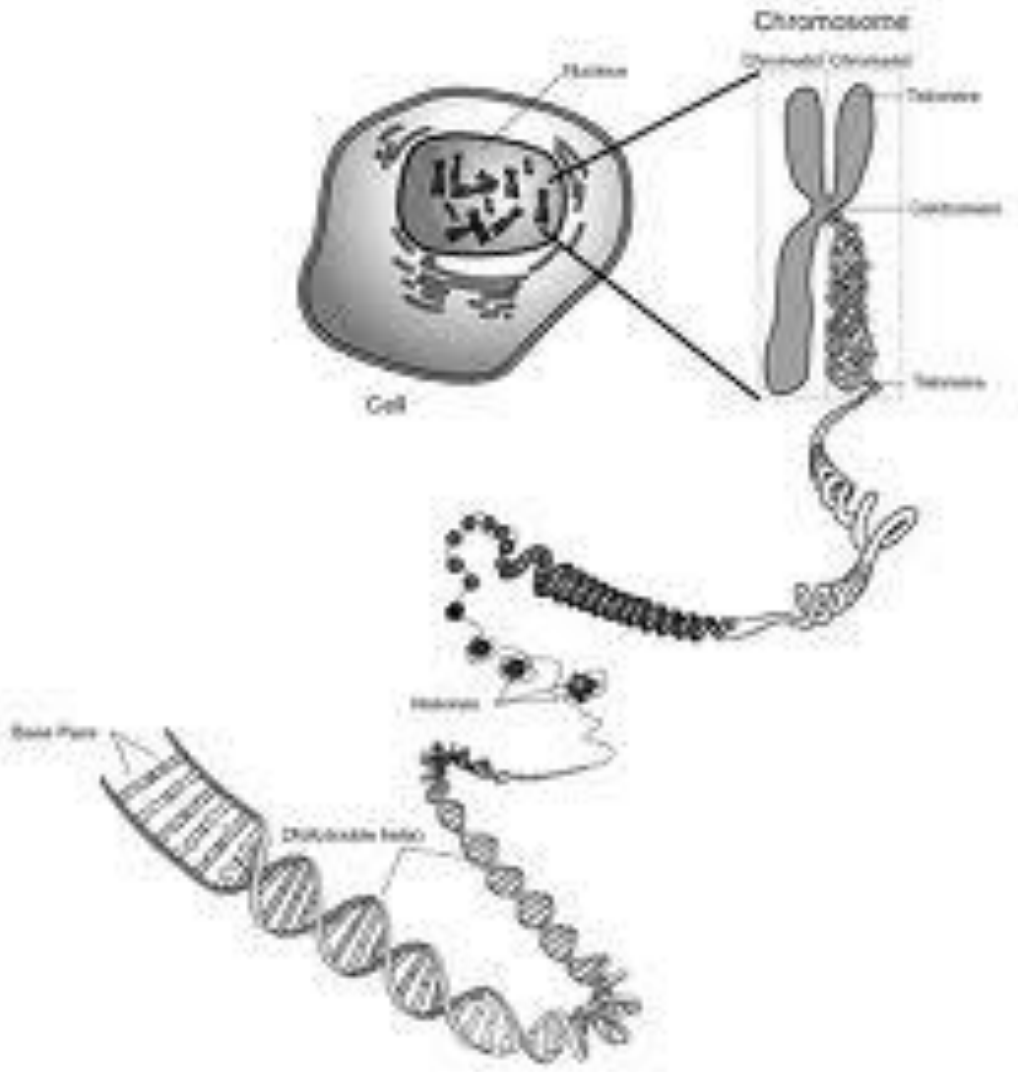


purines — adenine (A), guanine (G)

pyrimidines — thymine/uracil (T/U), cytosine (C)

complementary base pairing: A-T C-G

double helix



only present during cell division

chromatin form – present in active cell

Requirements for a hereditary material

1. ability to carry information and control protein synthesis
2. ability to replicate accurately
3. capable of variation

1.How information controlling protein synthesis is carried

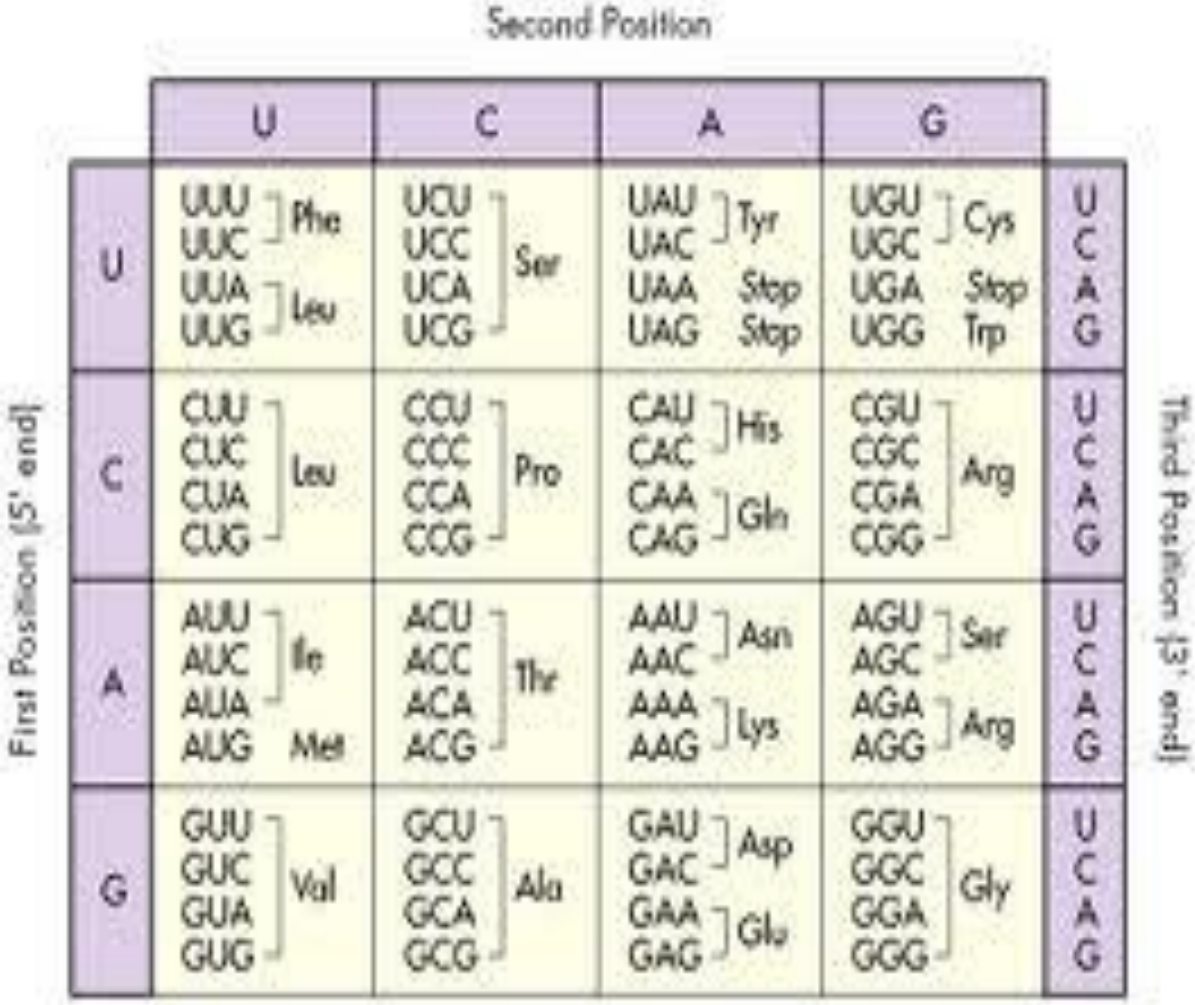
genetic code

- universal
- triplets of nucleotides code for single amino acids

Why a triplet?

only 4 bases 20 amino acids to code for

4^3 ⟶ 64 possible codes start stop 'wobble'

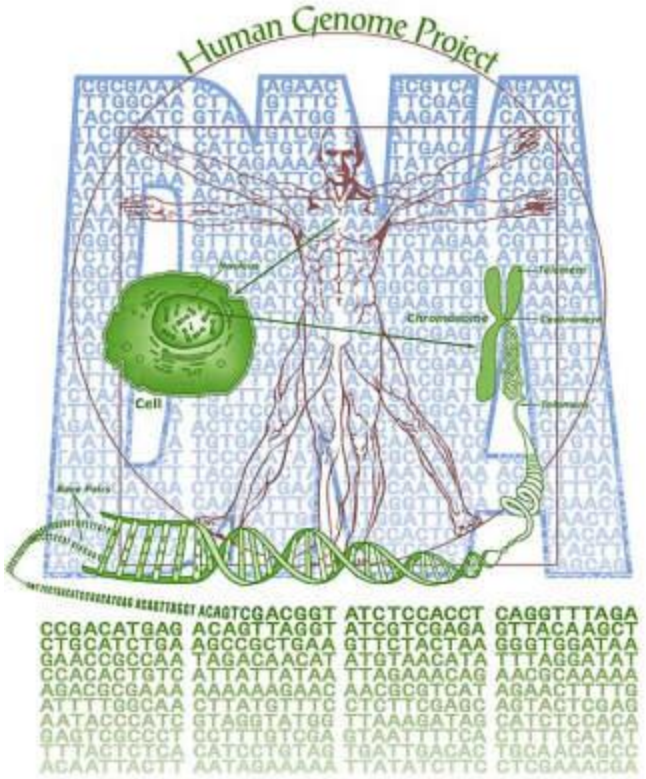

Second Position
U
C
A
G
First Position (5' end)
Third Position (3' end)
U
UUU Phe
UUC Phe
UUA Leu
UUG Leu
UCU Ser
UCC Ser
UCA Ser
UCG Ser
UAU Tyr
UAC Tyr
UAA Stop
UAG Stop
UGU Cys
UGC Cys
UGA Stop
UGG Trp
C
CUU Leu
CUC Leu
CUA Leu
CUG Leu
CCU Pro
CCC Pro
CCA Pro
CCG Pro
CAU His
CAC His
CAA Gln
CAG Gln
CGU Arg
CGC Arg
CGA Arg
CGG Arg
A
AUU Ile
AUC Ile
AUA Ile
AUG Met
ACU Thr
ACC Thr
ACA Thr
ACG Thr
AAU Asn
AAC Asn
AAA Lys
AAG Lys
AGU Ser
AGC Ser
AGA Arg
AGG Arg
G
GUU Val
GUC Val
GUA Val
GUG Val
GCU Ala
GCC Ala
GCA Ala
GCG Ala
GAU Asp
GAC Asp
GAA Glu
GAG Glu
GGU Gly
GGC Gly
GGA Gly
GGG Gly

Human genome

- 3 billion base pairs
  (3000 books, 500 pages each)

- completely sequenced
  1 error/100,000 bp

- estimated 22,000 genes (June2011)
  sequenced all protein kinases
  all transcription factors



- ~500 species sequenced

recently: 'chocolate' tree, strawberry (International Strawberry Sequencing Consortium)

human/human genomes 99.9% identical
human/chimp genomes 98.7% identical
human/daffodil genomes 35% identical

haplotype map still being finessed

- haplotypes

  small DNA regions

  each inherited intact

  - alleles in haplotype are in linkage disequilibrium

  vary across human populations

  allow imputation

genome only changes if mutations occur

does not vary cell to cell within one body barring somatic mutations

Other ….omes being worked on

vary organ to organ, cell to cell and temporally within one organism

- epigenome expression pattern (methylation) of the genome

most variation (80%) is influenced by genome sequence (genotype) itself
ie.functional effects of genetic variation extend into epigenetics

- variome all genetic variations that cause human disease

(China funding 25%, equivalent to world population %)

- proteome all proteins able to be synthesized by a genome

- transcriptome all RNA molecules able to be synthesized

2011 – comprehensive profile of human mitochondrial transcriptome

2012 - an anatomically comprehensive atlas of the adult human brain transcriptome

- metabonome all metabolically regulated elements

sex differences more than realized: 102 of 131 metabolites studied so far

microbiome

- all microbes present on/in human bodies
- bacteria, fungi, viruses, protists
- most found in digestive tract (100 trillion cells, 1000 species, 3-4lbs))
- 90% of protein-producing cells in the body are microbial
- 99% of functional genes are microbial - who inhabits who?
- 23000 human genes, 8million microbiome genes
- no 2 people have identical microbiomes

recent survey of salivary bacteria (Stahringer et al, 2012 Genome Research) showed no greater similarity MZ versus DZ through early adulthood, shared e accounts for familiarity seen

- different patterns of gut microbiota seen in lean versus obese humans
- transfer of gut bacteria from obese to germ-free mice produced 2 times increase in weight as transfer from lean mice even though same food eaten
- gut bacteria also implicated in risk for diabetes 2
- possibly depression , cancer

Bacteria and behaviour Gut instinct

Tantalising evidence that intestinal bacteria can influence mood

Sep 3rd 2011 | from the print edition

A GOOD way to make yourself unpopular at dinner parties is to point out that a typical person is, from a microbiologist's perspective, a walking, talking Petri dish. An extraordinary profusion of microscopic critters inhabit every crack and crevice of the typical human, so many that they probably outnumber the cells of the body upon and within which they dwell.

Happily, these microbes are mostly harmless. Some of them, particularly those that live in the gut, are positively beneficial, helping with digestion and keeping the intestines in good working order. That is no surprise—bacteria as much as people have an interest in keeping their homes in sound condition. What is surprising is the small but growing body of evidence which suggests that bacteria dwelling in the gut can affect the brain, too, and thereby influence an individual's mood and behaviour. The most recent paper on the topic, published this week in the *Proceedings of the National Academy of Sciences*, reports (like much of the research in this field) on results in mice.

The researchers, led by Javier Bravo of University College, Cork, split their rodent subjects into two groups. One lot were fed a special broth containing *Lactobacillus rhamnosus*, a gut-dwelling bacterium often found in yogurt and other dairy products. The others were fed an ordinary diet, not fortified with microbes.

The team then subjected the mice to a battery of tests that are used routinely to measure the emotional states of rodents. Most (though not all) of these tests showed significant differences between the two groups of animals.

One test featured a maze that had both enclosed and open tunnels. The researchers found that the bacterially boosted mice ventured out into the open twice as often as the control mice, which they interpreted to mean that these rodents were more confident and less anxious than those not fed *Lactobacillus*.

In another test the animals were made to swim in a container from which they could not escape. Bacteria-fed mice attempted to swim for longer than the others before they gave up and had to be rescued. Such persistence is usually interpreted by students of rodent behaviour as evidence of a more positive mood.

Direct measurements of the animals' brains supported the behavioural results. Levels of corticosterone, a stress hormone, were markedly lower in the bacteria-fed mice than they were in the control group when both groups were exposed to stressful situations. The number of receptors for gamma-aminobutyric acid, a natural chemical messenger that helps dampen the activity of certain nerve cells, varied in statistically significant ways between the brains of the two groups, with more in some parts of the treated animals' brains and fewer in others. Most intriguing of all, when Dr Bravo cut the animals' vagus nerves—which transmit signals between the gut and the brain—the differences between the groups vanished.

The idea that gut-dwelling microbes can affect an animal's state of mind may strike some people as outlandish, and there are certainly loose ends still to be tied up. Beyond their evidence that the vagus nerve is crucial to the relationship, for example, Dr Bravo and his colleagues do not yet know the precise mechanisms

Nature Reviews Neuroscience | AOP, published online 14 September 2011; doi:10.1038/nrn3115

BEHAVIOUR

'Chillax' with probiotics

> Chronic administration of *L. rhamnosus* (JB-1) reduced anxiety-like behaviour

There is increasing evidence that gut microflora can influence the brain, but the mechanistic basis for these effects is unknown. A new study by Bravo *et al.* shows that chronic administration of probiotic (or 'good') bacteria in healthy mice reduces levels of anxiety and depression-like behaviour, and induces changes in the GABAergic system in regions of the brain that are known to be involved in these behaviours.

The authors chronically treated healthy mice with the potentially probiotic strain of the bacterium *Lactobacillus rhamnosus* (JB-1). Chronic administration of *L. rhamnosus* (JB-1) reduced anxiety-like behaviour in an elevated plus maze, increased cue- and context-dependent freezing responses in the recall phase of a fear conditioning paradigm, and decreased the time spent immobile in a forced swim test. In addition, stress-induced plasma corticosterone levels were lower in treated mice. These behavioural changes are indicative of reduced anxiety, increased fear memory and reduced depression-like behaviour.

The GABAergic system is important in the regulation of behaviour, and the authors therefore investigated whether the behavioural effects of chronic administration of *L. rhamnosus* (JB-1) might be linked to changes in this system in brain areas that are involved in these behaviours. Mice that had received *L. rhamnosus* (JB-1) showed alterations in GABA receptor subunit mRNA expression as assessed by *in situ* hybridization. Specifically, chronic *L. rhamnosus* (JB-1) administration decreased expression of GABA type B ($GABA_B$) subunit 1 isoform b ($GABA_{B1b}$) mRNA in the amygdala and hippocampus, and increased it in cortical areas. Furthermore, it reduced expression of $GABA_{A\alpha2}$ receptor mRNA expression in amygdala and cortical areas, whereas levels were increased in the hippocampus. $GABA_{A\alpha1}$ mRNA levels were reduced in the amygdala and hippocampus, suggesting that probiotics might enhance responses to stressful situations through alterations in GABAergic function.

The most likely conduit for information from the gut to the brain is the vagus nerve, and the authors tested whether the effect of *L. rhamnosus* (JB-1) might be mediated by this nerve. They found that vagotomy prevented the anxiolytic and antidepressant effect of chronic *L. rhamnosus* (JB-1) ingestion. It also prevented the alterations in $GABA_{A\alpha2}$ mRNA expression in the amygdala. Although the authors did not assess whether behavioural changes were correlated with changes in mRNA expression levels, they suggest that modifications in the GABAergic system by *L. rhamnosus* (JB-1) treatment may underlie the effect of the treatment on behaviour.

These results show that a bacterium that is potentially probiotic influences brain physiology and function in healthy animals, and that at least some of these effects are mediated by the vagus nerve. There has been increasing interest in potential interactions between gut microbiota and the brain, and consumers of probiotic yoghurts may be encouraged by the findings of this study.

Sian Lewis

ORIGINAL RESEARCH PAPER Bravo, J. A. *et al.* Ingestion of Lactobacillus strain regulates emotional behavior and central GABA receptor expression in a mouse via the vagus nerve. *Proc. Natl Acad. Sci. USA* 29 Aug 2011 (doi:10.1073/pnas.1102999108)

FURTHER READING Mayer, E. A. Gut feelings: the emerging biology of gut–brain communication. *Nature Rev. Neurosci.* 12, 453–466 (2011)

Ingestion of *Lactobacillus* strain regulates emotional behavior and central GABA receptor expression in a mouse via the vagus nerve

Javier A. Bravo[a,1], Paul Forsythe[b,c,1], Marianne V. Chew[b], Emily Escaravage[b], Hélène M. Savignac[a,d], Timothy G. Dinan[a,e], John Bienenstock[b,f,2], and John F. Cryan[a,d,g,2]

[a]Laboratory of NeuroGastroenterology, Alimentary Pharmabiotic Centre, [d]School of Pharmacy, and Departments of [e]Psychiatry and [g]Anatomy, University College Cork, Cork, Ireland; [b]The McMaster Brain–Body Institute, St. Joseph's Healthcare, Hamilton, ON, Canada L8N 4A6; and Departments of [c]Medicine and [f]Pathology and Molecular Medicine, McMaster University, Hamilton, ON, Canada L8S 4L8

Edited by Todd R. Klaenhammer, North Carolina State University, Raleigh, NC, and approved July 27, 2011 (received for review February 27, 2011)

There is increasing, but largely indirect, evidence pointing to an effect of commensal gut microbiota on the central nervous system (CNS). However, it is unknown whether lactic acid bacteria such as *Lactobacillus rhamnosus* could have a direct effect on neurotransmitter receptors in the CNS in normal, healthy animals. GABA is the main CNS inhibitory neurotransmitter and is significantly involved in regulating many physiological and psychological processes. Alterations in central GABA receptor expression are implicated in the pathogenesis of anxiety and depression, which are highly comorbid with functional bowel disorders. In this work, we show that chronic treatment with *L. rhamnosus* (*JB-1*) induced region-dependent alterations in $GABA_{1b}$ mRNA in the brain with increases in cortical regions (cingulate and prelimbic) and concomitant reductions in expression in the hippocampus, amygdala, and *locus coeruleus*, in comparison with control-fed mice. In addition, *L. rhamnosus* (*JB-1*) reduced $GABA_{A\alpha2}$ mRNA expression in the prefrontal cortex and amygdala, but increased $GABA_{A\alpha2}$ in the hippocampus. Importantly, *L. rhamnosus* (*JB-1*) reduced stress-induced corticosterone and anxiety- and depression-related behavior. Moreover, the neurochemical and behavioral effects were not found in vagotomized mice, identifying the vagus as a major modulatory constitutive communication pathway between the bacteria exposed to the gut and the brain. Together, these findings highlight the important role of bacteria in the bidirectional communication of the gut–brain axis and suggest that certain organisms may prove to be useful therapeutic adjuncts in stress-related disorders such as anxiety and depression.

brain-gut axis | irritable bowel syndrome | probiotic | fear conditioning | cognition

There is increasing evidence suggesting an interaction between the intestinal microbiota, the gut, and the central nervous system (CNS) in what is recognized as the microbiome–gut–brain axis (1–4). Studies in rodents have implicated dysregulation of this axis in functional bowel disorders, including irritable bowel syndrome. Indeed, visceral perception in rodents can be affected by alterations in gut microbiota (5). Moreover, it has been shown that the absence and/or modification of the gut microflora in mice affects the hypothalamic–pituitary–adrenal (HPA) axis response to stress (6, 7) and anxiety behavior (8, 9), which is important given the high comorbidity between functional gastrointestinal disorders and stress-related psychiatric disorders, such as anxiety and depression (10). In addition, pathogenic bacteria in rodents can induce anxiety-like behaviors, which are mediated via vagal afferents (9, 11).

GABA is the main inhibitory neurotransmitter of the CNS, the effects of which are mediated through two major classes of receptors—the ionotropic $GABA_A$ receptors, which exist as a number of subtypes formed by the coassembly of different subunits (α, β, and γ subunits; ref. 12), and the $GABA_B$ receptors, which are G protein coupled and consist of a heterodimer made up of two subunits ($GABA_{B1}$ and $GABA_{B2}$), both of which are necessary for $GABA_B$ receptor functionality (13). These receptors are important pharmacological targets for clinically relevant antianxiety agents (e.g., benzodiazepines acting on $GABA_A$ receptors), and alterations in the GABAergic system have important roles in the development of stress-related psychiatric conditions.

Probiotic bacteria are living organisms that can inhabit the gut and contribute to the health of the host (14). Accumulating clinical evidence suggests that probiotics can modulate the stress response and improve mood and anxiety symptoms in patients with chronic fatigue and irritable bowel syndrome (15, 16). One such organism is *Lactobacillus rhamnosus* (*JB-1*), which has been demonstrated to modulate the immune system because it prevents the induction of IL-8 by TNF-α in human colon epithelial cell lines (T84 and HT-29) (17) and modulates inflammation through the generation of regulatory T cells (18). Moreover, it inhibits the cardio–autonomic response to colorectal distension (CRD) in rats (19), reduces CRD-induced dorsal root ganglia excitability (20), and affects small intestine motility (21).

It is currently unclear whether potential probiotics such as *L. rhamnosus* (*JB-1*) could affect brain function, especially in normal, healthy animals. To this end, we sought to assess whether this bacteria could mediate direct effects on the GABAergic system. In parallel, behaviors relevant to GABAergic neurotransmission and the stress response were assessed subsequent to *L. rhamnosus* (*JB-1*) administration. Finally, the role of the vagus nerve in mediating such effects was also investigated by examining these parameters in subdiaphragmatically vagotomized mice.

Results

Behavioral Effects of *L. rhamnosus* (*JB-1*) Administration. A battery of behavioral tests relevant to anxiety and depression was carried out. The stress-induced hyperthermia (SIH) and elevated plus maze (EPM) tests are widely used for assessing functional consequences of alterations in GABA neurotransmission (22, 23). Chronic administration of *L. rhamnosus* (*JB-1*) produced a nonsignificant reduction in SIH ($t = 1.567$, df = 34; $P = 0.1263$; Fig. 1*A*). On the EPM, animals treated with *L. rhamnosus* (*JB-1*) had a larger number of entries to the open arms than broth-fed animals, suggesting anxiolytic effects (open arm entry defined as all four paws entering the arms of the EPM apparatus) ($t = 4.662$, df = 34; $P < 0.001$; Fig. 1*A*). This effect is also reflected in the percentage of time spent in the open arms, although this observation did not reach statistical significance [broth v. *L. rhamnosus* (*JB-1*): 25.28 ± 6.67% vs. 38.36 ± 7.99%; $t = 1.267$, df = 34; $P = 0.2146$].

Author contributions: J.A.B., P.F., M.V.C., H.M.S., T.G.D., J.B., and J.F.C. designed research; J.A.B., P.F., M.V.C., E.E., and H.M.S. performed research; J.A.B., P.F., H.M.S., J.B., and J.F.C. analyzed data; and J.A.B., P.F., T.G.D., J.B., and J.F.C. wrote the paper.

The authors declare no conflict of interest.

This article is a PNAS Direct Submission.

[1]J.A.B. and P.F. contributed equally to this work.

[2]To whom correspondence may be addressed. E-mail: bienens@mcmaster.ca or j.cryan@ucc.ie.

This article contains supporting information online at www.pnas.org/lookup/suppl/doi:10.1073/pnas.1102999108/-/DCSupplemental.

- less than 2% of genome is protein-coding (exon)
- produces ½-1.5m proteins

most genes express multiple mRNA isoforms through eg. alternative splicing, selection of alternative 5`, 3` ends

brain – alternative splicing important in development + normal function in maturity

- 98%? much of this used to be called 'junk' DNA, now more respectfully called non-coding, or 'dark matter'

ENCODE ENCyclopedia Of DNA Elements project

- aims to assign functions to all DNA
- recently (Sept, 2012) released research assigning roles to 80% of DNA
  - ¾ of the genome is involved in making RNA which helps regulate gene activity
  - 4 million sites mapped where protein binds to DNA to regulate gene function (acts as 'switches' to turn genes on and off)

Human genome and inherited disease

- 3000 (out of +20,000) human genes known to have at least 1 mutation that causes an inherited disease
- Information kept on NCBI (National Center for Biotechnology Information) now also Human Variome Project
- 1/3 to ½ of all genes are expressed in the brain - more expressed than in any other organ

  reflected in large number of neurogenetic disorders

  >30% of Mendelian diseases have neurological manifestations

accurate diagnosis & counseling possible for single-gene causes with known genome location

Most genetic disorders, however, show any or several of the following:

genetic heterogeneity, variable expression, incomplete penetrance, anticipation, phenocopies, imprinting even mitochondrial inheritance

there may also be undetected epistasis and GxE interactions

- all complicate relating phenotype to genotype

Protein synthesis
- how the information coded into DNA is used

1. transcription

   DNA code is transcribed to form mRNA molecule

   RNA polymerase

2. RNA processing

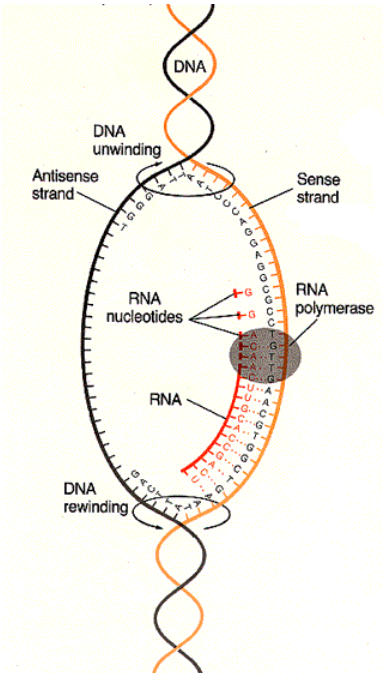
   introns spliced out leaving exons

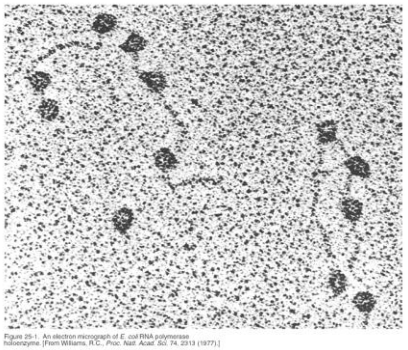
alternative splicing (+1/2 of all genes)

3. translation

   mRNA code is translated into sequence of amino acids to form polypeptide

microarrays – used to study expression of many genes at once (transcriptome)





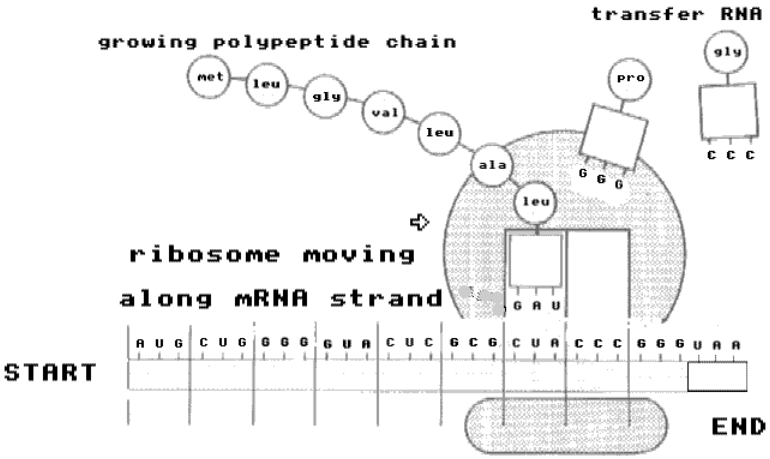
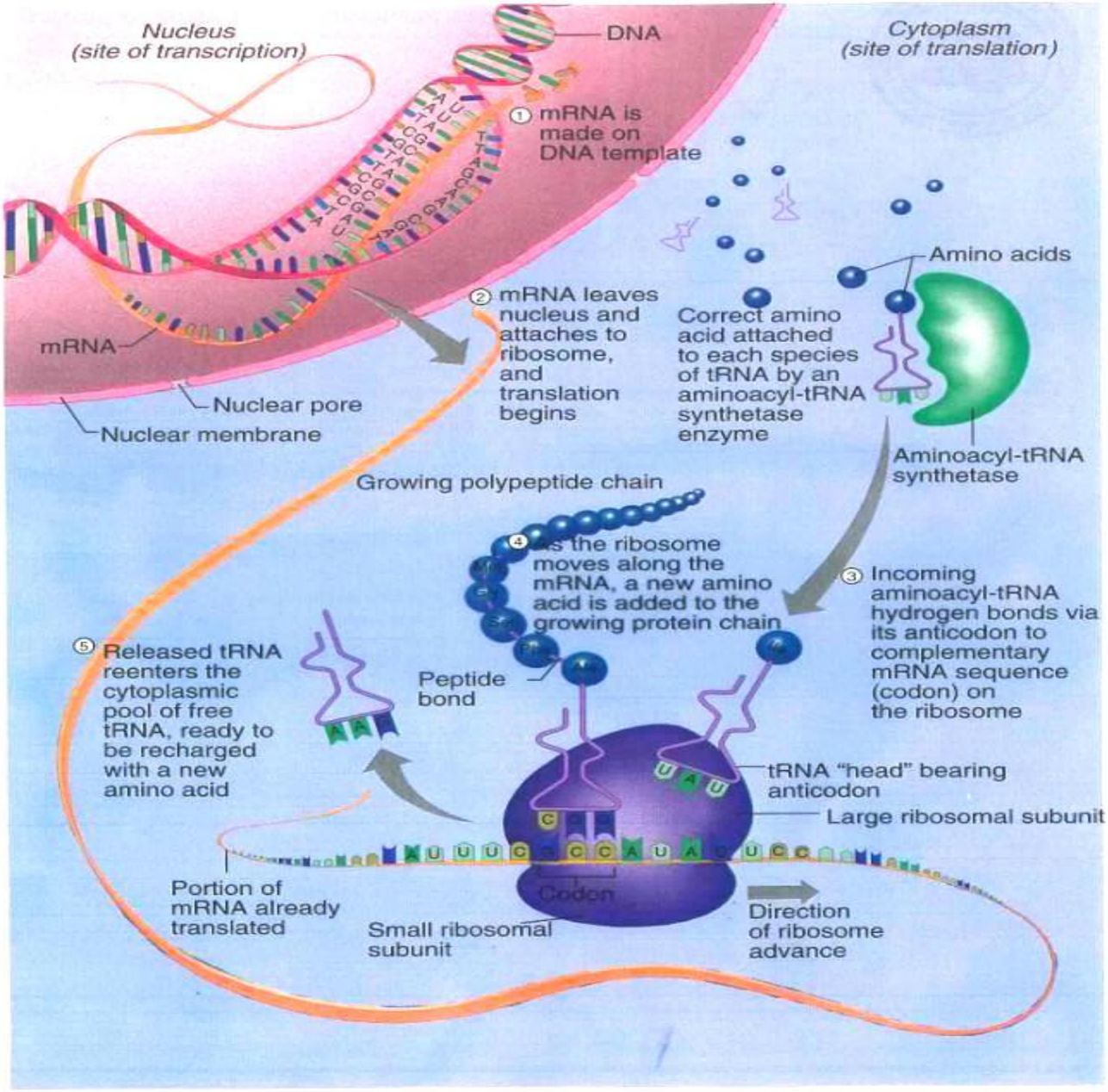


Figure 3.32: Proteinsynthesis, page 105



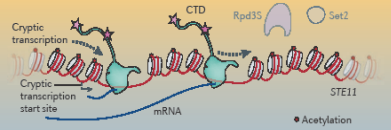
nature REVIEWS GENETICS

Chromatin remodelling and the transcription cycle

Vikki M. Weake and Jerry L. Workman

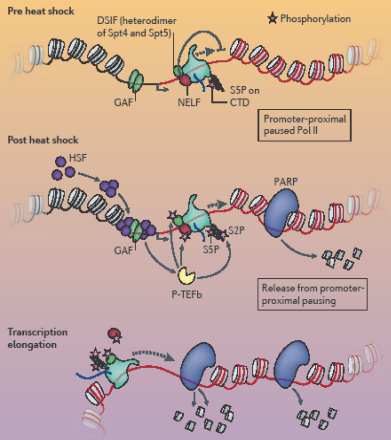
Transcription by RNA polymerase II (Pol II) occurs in the context of chromatin within a eukaryotic cell. Chromatin is generally inhibitory to transcription, so a variety of mechanisms are required to activate transcription from a nucleosomal template. One of the first steps is that large co-activator complexes interact with small activator proteins to identify gene promoters that are ready to be transcribed. Nucleosome remodelling complexes that use energy from ATP to move or displace nucleosomes from DNA facilitate the recruitment and assembly of these complexes on the promoter and enable rapid gene activation. Even during transcription elongation, nucleosomes must be removed for efficient passage of the polymerase. Furthermore, these same nucleosomes must be reassembled rapidly and modified appropriately following passage of the polymerase to prevent inappropriate initiation of transcription from promoter-like elements within the coding region.

Example of chromatin regulation during elongation: *STE11* in yeast



In yeast, loss of the histone deacetylase complex Rpd3S, or the H3K36 methyltransferase Set2 results in hyperacetylation of the coding region of genes such as *STE11*. Promoter-like regions within the coding region are then able to recruit Pol II and components of the general transcription machinery, and transcription can be initiated inappropriately at these cryptic initiation sites. Thus, proper regulation of histone assembly, disassembly and modifications are critical to control transcription on a chromatin template.

Example of regulation by polymerase pausing: heat shock genes in *Drosophila melanogaster*

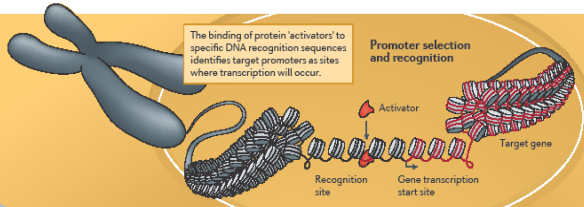


Heat shock genes in Drosophila melanogaster are rate-limited during early elongation. Prior to heat shock, GAF, co-activators and the GTFs are bound at *Hsp70* and Pol II is present at the promoter-proximal pause site, where it sits in a poised state ready to resume productive elongation. Heat shock induces trimerization of the transcription factor HSF, which then binds to the promoter of *Hsp70*. Binding of HSF is required, but is not sufficient, to recruit the activating kinase P-TEFb, which phosphorylates the inhibitory factors NELF and DSIF, as well as serine 2 of the CTD, resulting in release of Pol II into productive transcription elongation. PARP catalyses formation of ADP-ribose polymers, and along with HSF and GAF is required for nucleosome loss at *Hsp70* following heat shock. Nucleosome loss preceeds the passage of Pol II and facilitates gene activation.

During transcription elongation, the phosphorylated residues on the CTD provide binding sites for chromatin modifiers such as SETD2 (Set2 in yeast and flies), which methylates H3K36. Efficient transcription requires chromatin remodelling by complexes such as SWI/SNF and RSC, and histone chaperones such as the FACT complex and SPT6. Nucleosomes must be displaced ahead of Pol II and reassembled following its passage. Histone modifications are carefully regulated to prevent inappropriate transcription initiation from within the coding region of genes.

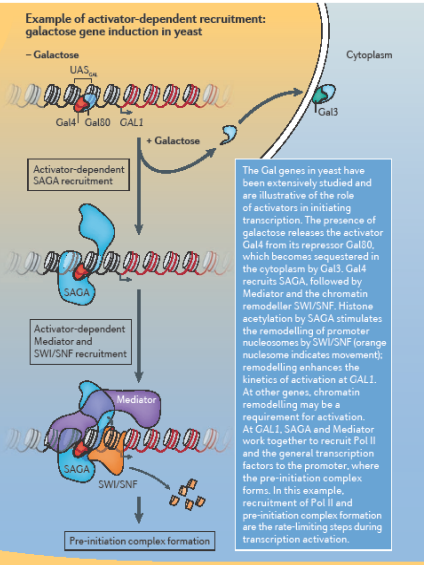
The binding of protein 'activators' to specific DNA recognition sequences identifies target promoters as sites where transcription will occur.

Promoter selection and recognition



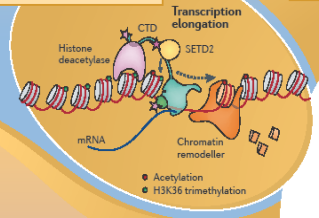
Activators recruit large multi-subunit co-activators such as Mediator, the histone acetyltransferase complex SAGA, and chromatin remodelling complexes (such as SWI/SNF) that use the energy from ATP to move or displace nucleosomes at the promoter.

Example of activator-dependent recruitment: galactose gene induction in yeast



The Gal genes in yeast have been extensively studied and are illustrative of the role of activators in initiating transcription. The presence of galactose releases the activator Gal4 from its repressor Gal80, which becomes sequestered in the cytoplasm by Gal3. Gal4 recruits SAGA, followed by Mediator and the chromatin remodeller SWI/SNF. Histone acetylation by SAGA stimulates the remodelling of promoter nucleosomes by SWI/SNF (orange nucleosome indicates movement); remodelling enhances the kinetics of activation at *GAL1*. At other genes, chromatin remodelling may be a requirement for activation. At *GAL1*, SAGA and Mediator work together to recruit Pol II and the general transcription factors to the promoter, where the pre-initiation complex forms. In this example, recruitment of Pol II and pre-initiation complex formation are the rate-limiting steps during transcription activation.

Transcription elongation

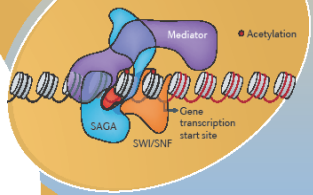


Termination and reinitiation

The transcription cycle

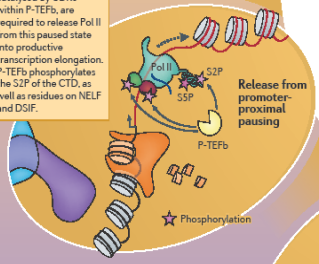
The central cycle shows some of the main steps in transcription. At different genes, different stages of this cycle can be the key regulatory (rate-determining) steps. Many aspects are conserved among species (in the central panels, human nomenclature is mainly used). Only a selection of proteins and chromatin modifications are shown to illustrate the sequence of events. The side panels show examples of particular genes or provide more detail for certain stages.

Activator-dependent recruitment of co-activators

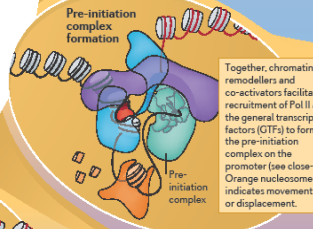


A second series of phosphorylation events, catalysed by CDK9 within P-TEFb, are required to release Pol II from this paused state into productive transcription elongation. P-TEFb phosphorylates the S2P of the CTD, as well as residues on NELF and DSIF.

Release from promoter-proximal pausing

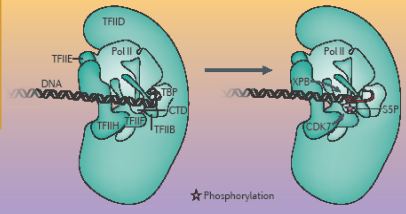


Pre-initiation complex formation

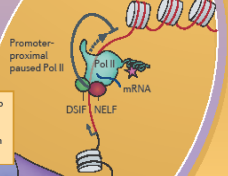


Together, chromatin remodellers and co-activators facilitate recruitment of Pol II and the general transcription factors (GTFs) to form the pre-initiation complex on the promoter (see close-up). Orange nucleosome indicates movement or displacement.

The pre-initiation complex prior to promoter clearance

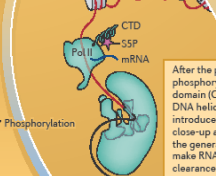


Promoter-proximal pausing



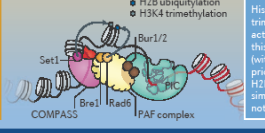
At some genes, after Pol II transcribes a short distance into the gene, it pauses at a region of DNA known as the promoter-proximal pause site. Pol II is prevented from moving forward from this region by inhibitory factors such as NELF and DSIF. (Note: NELF is not present in yeast.)

Promoter clearance



After the pre-initiation complex has formed, CDK7 within TFIIH phosphorylates the serine-5 position (S5P) within the carboxy-terminal domain (CTD) of the largest subunit of Pol II. Around the same time, the DNA helicase XPB unwinds 11–15 bases of DNA at the promoter to introduce a single-stranded template into the active site of Pol II (see close-up above). Transcription begins as Pol II dissociates from many of the general transcription factors, clears the promoter and begins to make RNA. During pre-initiation complex formation and promoter clearance, several different histone modifications are deposited on nucleosomes at the promoter, including H3K4 trimethylation and H2B monoubiquitylation (see side panel).

Sequential histone modifications occur at promoters



Histone H3 lysine 4 (H3K4) trimethylation is associated with active transcription. In yeast, this mark is deposited by Set1 (within COMPASS) and requires prior ubiquitylation of histone H2B by Rad6 and Bre1. For simplicity, these proteins are not shown in other panels.

References

Fuda, N. J., Ardehali, M. B. & Lis, J. T. Defining mechanisms that regulate RNA polymerase II transcription in vivo. *Nature* **461**, 186–192 (2009) | Weake, V. M. & Workman, J. L. Inducible gene expression: diverse regulatory mechanisms. *Nature Rev. Genet.* **11**, 426–437 (2010) | Kornberg, R. D. The molecular basis of eukaryotic transcription. *Proc. Natl Acad. Sci. USA* **104**, 12955–12961 (2007) | Orphanides, G. & Reinberg, D. A unified theory of gene expression. *Cell* **108**, 439–451 (2002) | Ptashne, M. & Gann, A. Transcriptional activation by recruitment. *Nature* **386**, 569–577 (1997) | Roeder, R. G. Transcriptional regulation and the role of diverse coactivators in animal cells. *FEBS Lett.* **579**, 909–915 (2005)

Abbreviations

CDK, cell division protein kinase; COMPASS, complex proteins associated with Set1; DSIF, DRB sensitivity-inducing factor; GAF, GAGA factor; HSF, heat shock factor; *Hsp70*, heat shock protein 70; NELF, negative elongation factor; PARP, poly(ADP)-ribose polymerase; P-TEFb, positive transcription elongation factor b; SWI/SNF, switch/sucrose non-fermentable; TBP, TATA box binding protein; TFII, transcription factor II; Tra1, transcription associated protein 1; UAS, upstream activating sequence

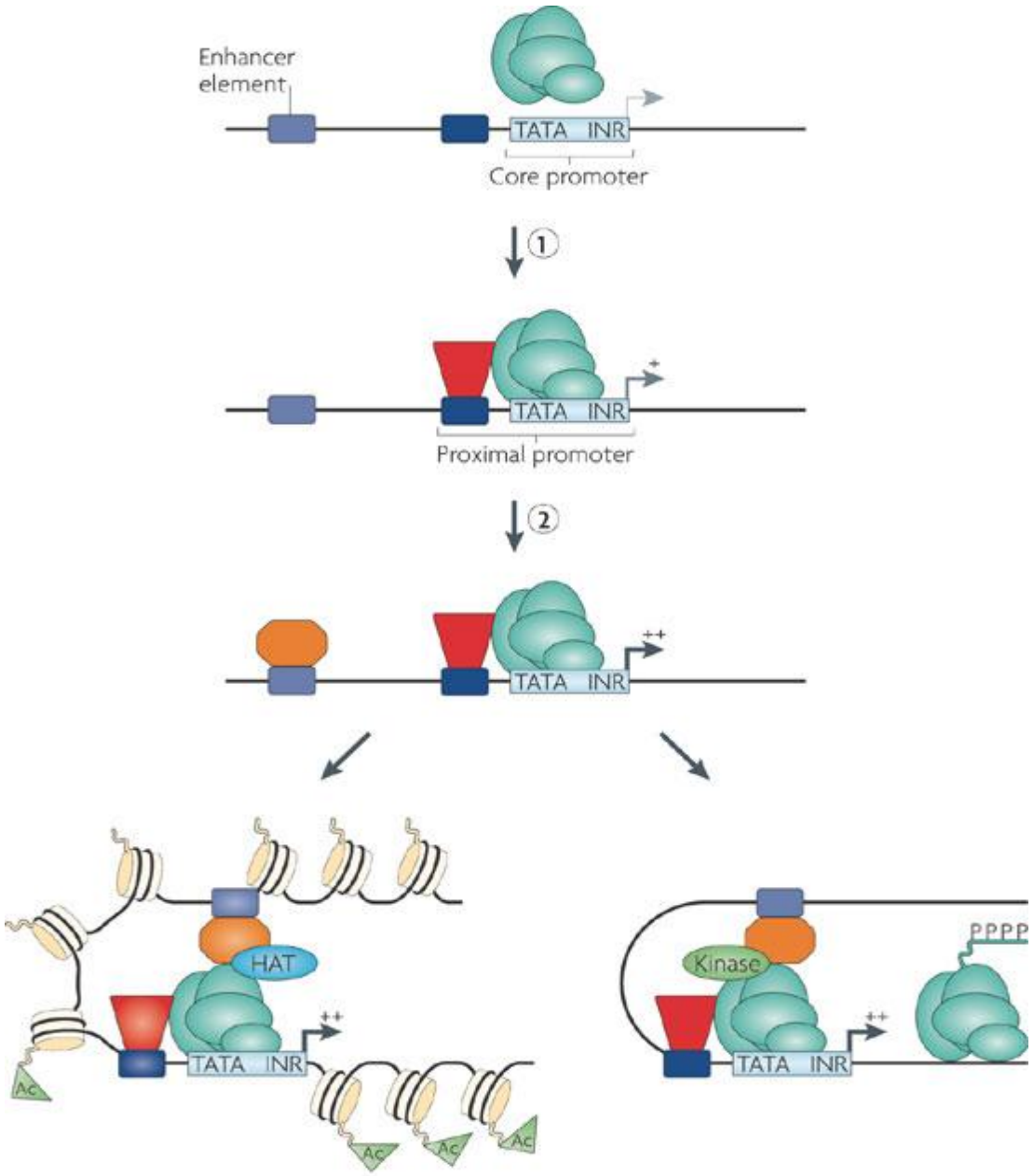
Acknowledgements

The authors are at the Stowers Institute for Medical Research, Kansas City, Missouri, USA. They thank members of the Workman laboratory for helpful comments and suggestions during preparation of this poster.

Edited by Mary Muers; copyedited by Lewis Packwood; designed by Patrick Morgan.

http://www.nature.com/nrg/posters/remodelling

General transcription factors (green ovals) bind to core promoter regions through recognition of common elements such as TATA boxes and initiators (INR). However, these elements on their own provide very low levels of transcriptional activity owing to unstable interactions of the general factors with the promoter region. Promoter activity can be increased (represented by +) by site-specific DNA-binding factors (red trapezoid) interacting with *cis* elements (dark blue box) in the proximal promoter region and stabilizing the recruitment of the transcriptional machinery through direct interaction of the site-specific factor and the general factors (step 1). Promoter activity can be further stimulated to higher levels by site-specific factors (orange octagon) binding to enhancers (step 2). The enhancer factors can stimulate transcription by (bottom left) recruiting a histone-modifying enzyme (for example, a histone acetyltransferase (HAT)) to create a more favourable chromatin environment for transcription (for example, by histone acetylation (Ac)) or by (bottom right) recruiting a kinase that can phosphorylate (P) the carboxy-terminal domain of RNA polymerase II and stimulate elongation



Nature Reviews Genetics **10**, 605-616 (September 2009) | doi:10.1038/nrg2636**Insights from genomic profiling of transcription factors**Peggy J. Farnham[1]

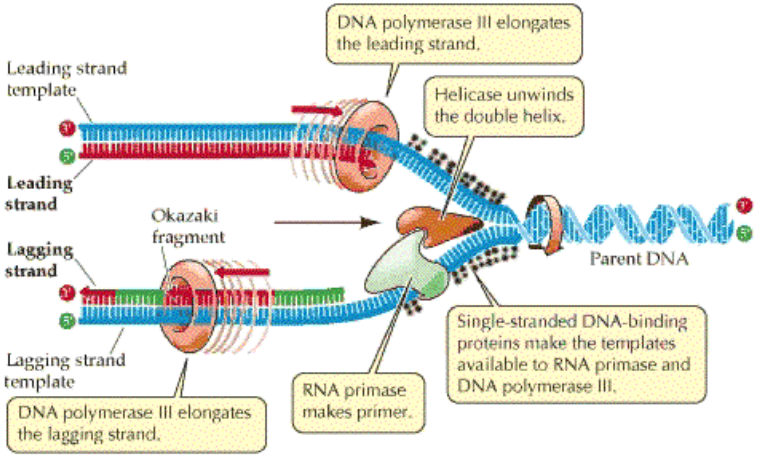
Serotonin-receptor (1A subtype) - amino acid sequence

DNA replication

- how DNA copies are produced

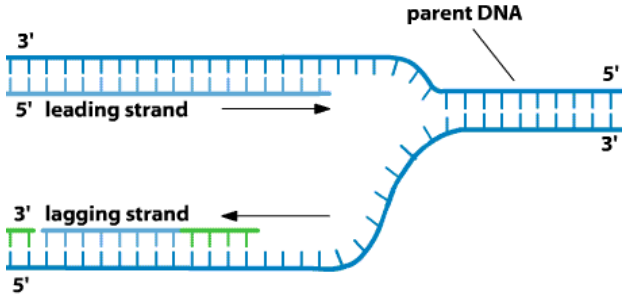
- occurs during S-phase of interphase

1. DNA double helix is unwound
2. strands are separated
3. DNA polymerase creates new strand on each template (original) strand



semi-conservative replication

http://www.youtube.com/watch?v=4PKjF7OumYo&eurl=http://io9.com/5142583/the-most-awesome-science-video-about-dna-ever-made



DNA sequence mutations

why DNA varies, makes evolution possible

- copying errors
  - somatic mutations - not passed on to offspring
  - germ-line mutations – passed on to offspring
- the only way new alleles are formed
- almost always deleterious

point mutations newly arising = de novo mutations

- single base-pair changes in nucleotide sequence
- commonly called SNPs – single nucleotide polymorphisms

Also possible:

DNA sequence changes of longer length eg triplet repeats

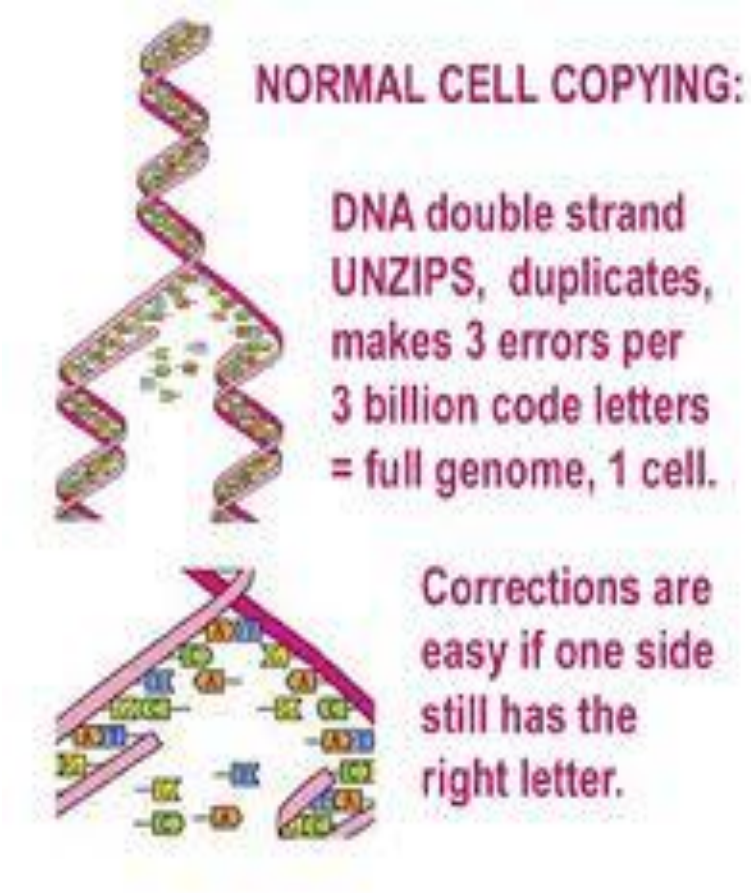
chromosome mutations – changes in number or structure of chromosomes

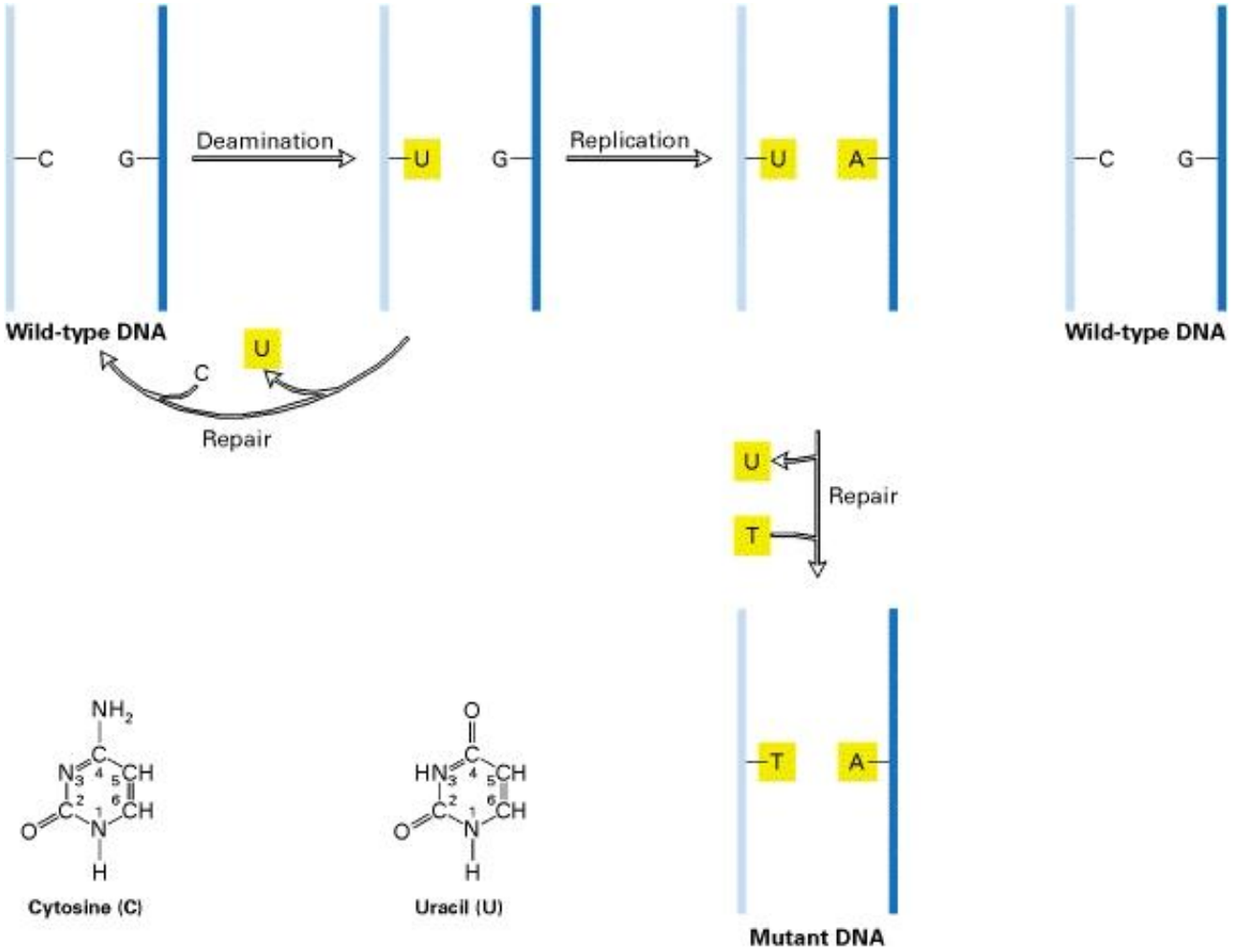
DNA polymerase
- prob. of mismatch per base pair per replication
= 10^{-5}

Proofreading function
- prob. of not correcting mismatch
= 10^{-2}

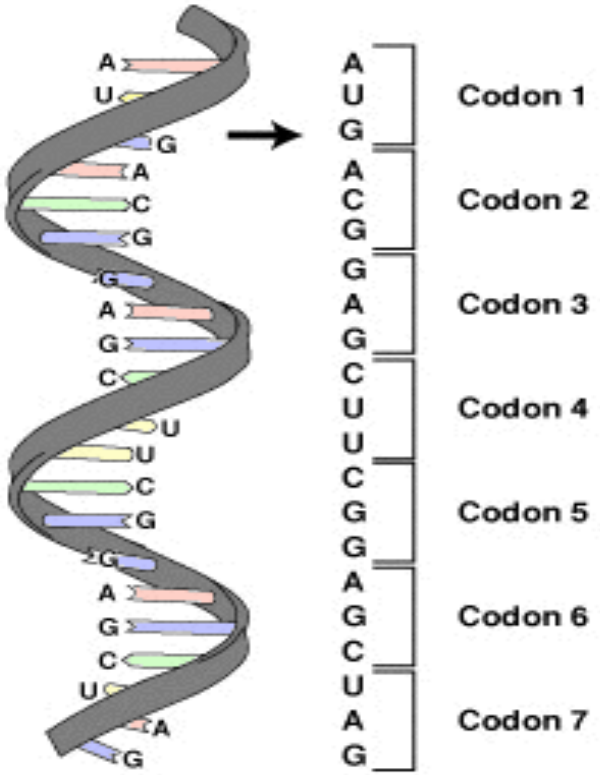
Postreplication mismatch repair
- prob. of not correcting mismatch
= 10^{-3}

Overall prob. of nucleotide substitution per base pair per replication
= $10^{-5} \times 10^{-2} \times 10^{-3}$
= 10^{-10}





Introduction of point mutation by deamination of cytosine



RNA

Ribonucleic acid

Point mutations

1. Substitutions

one base-pair is substituted for another

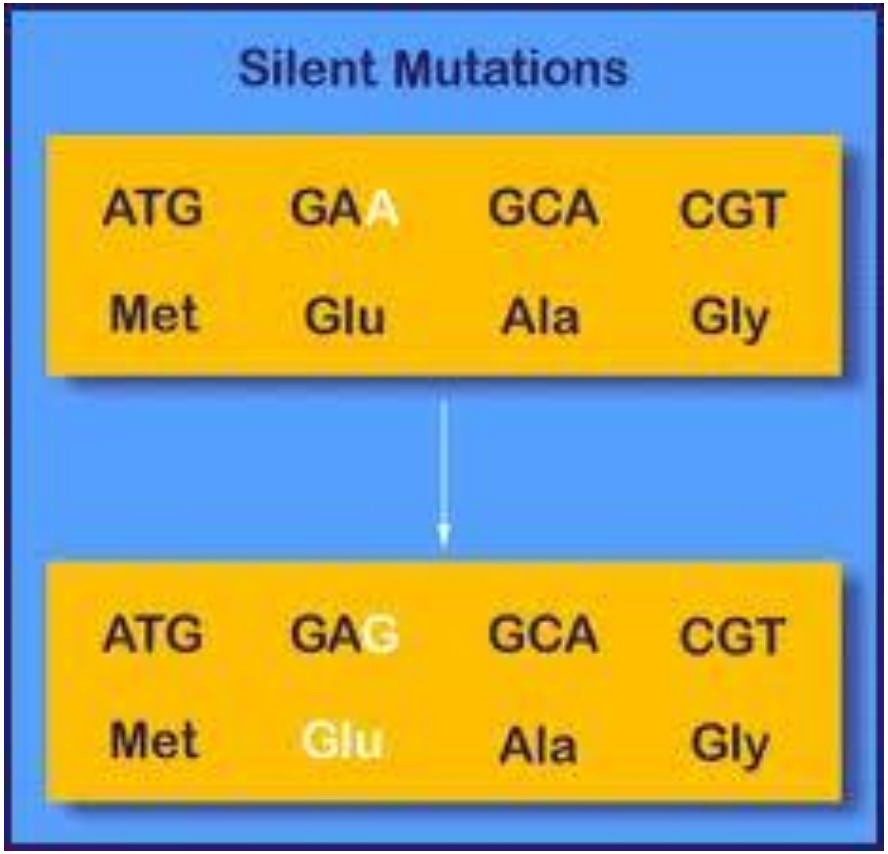
synonymous mutations (neutral, silent)

- base-pair substitution produces no change in amino acid sequence(synonymous) and/or no change in function (silent, neutral)

Tp53 tumor suppressor gene, codes for transcription factor that controls many genes important for regulating cell cycle

mutated in almost all cancer cells – some point mutations produce change in function

but >200 point mutations occur naturally that produce NO change in function or increase in cancer risk


Silent Mutations
ATG GAA GCA CGT
Met Glu Ala Gly
ATG GAG GCA CGT
Met Glu Ala Gly

Understanding the contribution of synonymous mutations to human disease

Zuben E. Sauna and Chava Kimchi-Sarfaty

Abstract | Synonymous mutations — sometimes called 'silent' mutations — are now widely acknowledged to be able to cause changes in protein expression, conformation and function. The recent increase in knowledge about the association of genetic variants with disease, particularly through genome-wide association studies, has revealed a substantial contribution of synonymous SNPs to human disease risk and other complex traits. Here we review current understanding of the extent to which synonymous mutations influence disease, the various molecular mechanisms that underlie these effects and the implications for future research and biomedical applications.

Splicing
The transcribed precursor RNA consists of exons (which encode amino acids) and introns, which must be edited out: splicing is the process by which this occurs.

Synonymous SNPs
(sSNPs). Single-nucleotide changes that do not result in a change in the amino acid in the translated protein.

Protein therapeutics
Proteins used in the treatment of human diseases that are purified from animal or human sources or, increasingly, manufactured by recombinant DNA technology.

Laboratory of Hemostasis, Division of Hematology, Center for Biologics Evaluation and Research, Food and Drug Administration, 29 Lincoln Drive, Bethesda, Maryland 20892, USA.
e-mails: zuben.sauna@fda.hhs.gov; chava.kimchi-sarfaty@fda.hhs.gov
doi:10.1038/nrg3061
Published online
31 August 2011

Rapid progress in our understanding of human genetic variation shows that many different types of genetic changes affect complex phenotypes, such as disease risk. Historically, much of the focus has been on mutations that change the amino acid sequence of a protein (non-synonymous mutations), but there is increasing awareness that other types of genetic variations can affect disease risk and treatment outcomes. Owing to the degeneracy of the genetic code, synonymous mutations occurring in the gene-coding regions do not change the amino acid composition of the encoded proteins. In addition, mutations in introns, 3′ and 5′UTRs and other non-coding regions do not alter amino acid sequences. Owing to the dogma that the structure (and therefore function) of proteins is determined by the amino acid sequence[1], synonymous mutations were, until recently, referred to as silent.

A corollary of this perception was that synonymous mutations would have no effect on the fitness of an organism and would be 'neutral' during evolution[2]. Two lines of evidence began to suggest that synonymous mutations could have functional consequences. The first was based on findings that, in many organisms, there is a codon usage bias vis-à-vis synonymous codons, suggesting that even synonymous codons were under evolutionary pressure (see REFS 3,4 for comprehensive Reviews on this subject, which is not discussed here). Second, advances in our understanding of protein synthesis and folding have led to discoveries that provide the mechanistic and conceptual framework to understand this phenomenon. Considerable evidence has accumulated over the past decade to show that synonymous mutations can result in aberrant mRNA splicing, which can lead to human disease[5]. Emerging evidence also suggests that synonymous SNPs (sSNPs) could affect mRNA stability and thus protein expression and enzymatic activity[6]. In addition, although a theoretical case for how codon bias could affect tertiary protein structure was presented several decades ago, it was only demonstrated in 2007 that sSNPs can affect protein conformation and have functional and clinical consequences[7].

Technological innovation in genotyping platforms[8–11] and the development of computational methods to analyse these data have resulted in dramatic advancements in genetic association studies over the past decade; studies that used to be limited to a small number of candidate genes now encompass the entire genome. These genome-wide association studies (GWASs), by taking a hypothesis-free approach, have made it possible to identify loci in genes that were not necessarily previously associated with the disease[12].

In this article, we have generated a compendium of human diseases or clinical conditions associated with synonymous mutations. We also discuss recent studies that are elucidating the mechanisms by which synonymous substitutions bring about changes in the phenotype by affecting splicing accuracy, translation fidelity, mRNA structure and protein folding. Finally, we discuss how an increased understanding of the effect of synonymous mutations could have an impact on future clinical applications, such as pharmacogenetics and protein therapeutics.

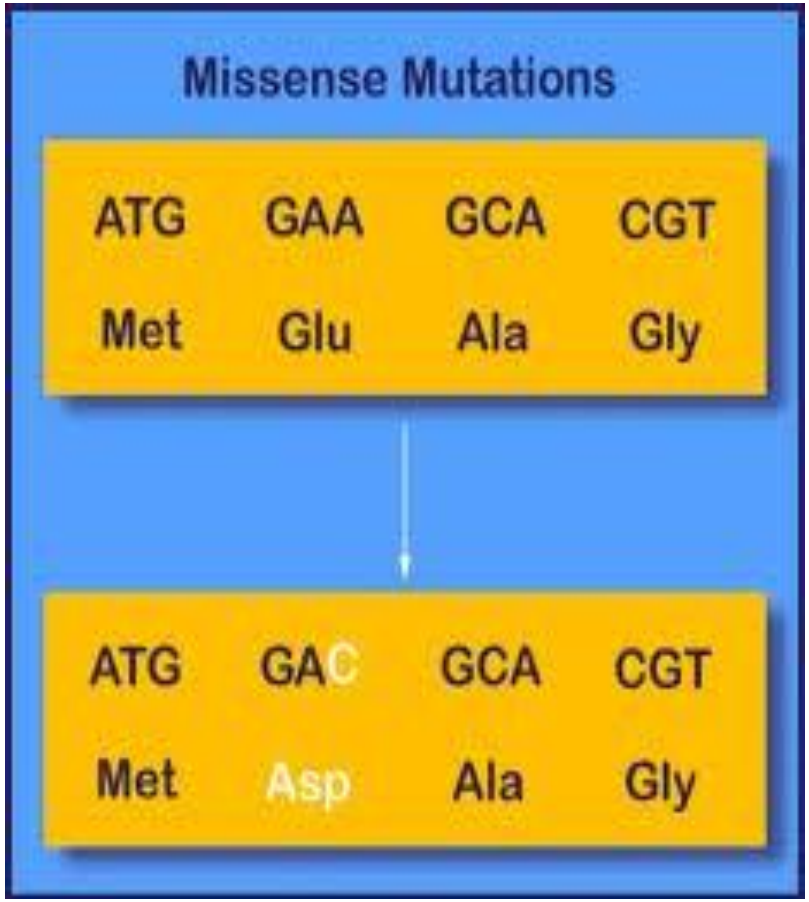
missense (non-synonymous) mutation

- base-pair substitution results in substitution of amino acid and change in function of protein product
- result is a functional polymorphism
- sickle cell cystic fibrosis

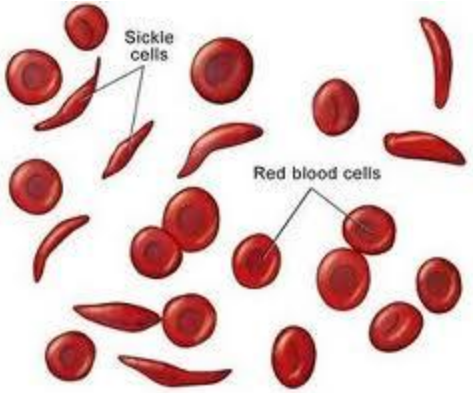
PKU

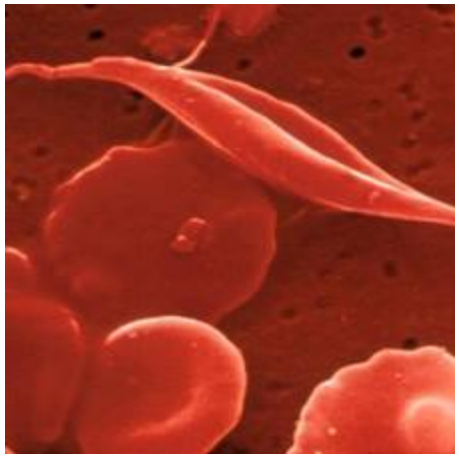
codon 1 AUG → GUG start → val no product

codon 408 CGG → UGG arg → trp low activity



Glutamic acid to asparagine substitution

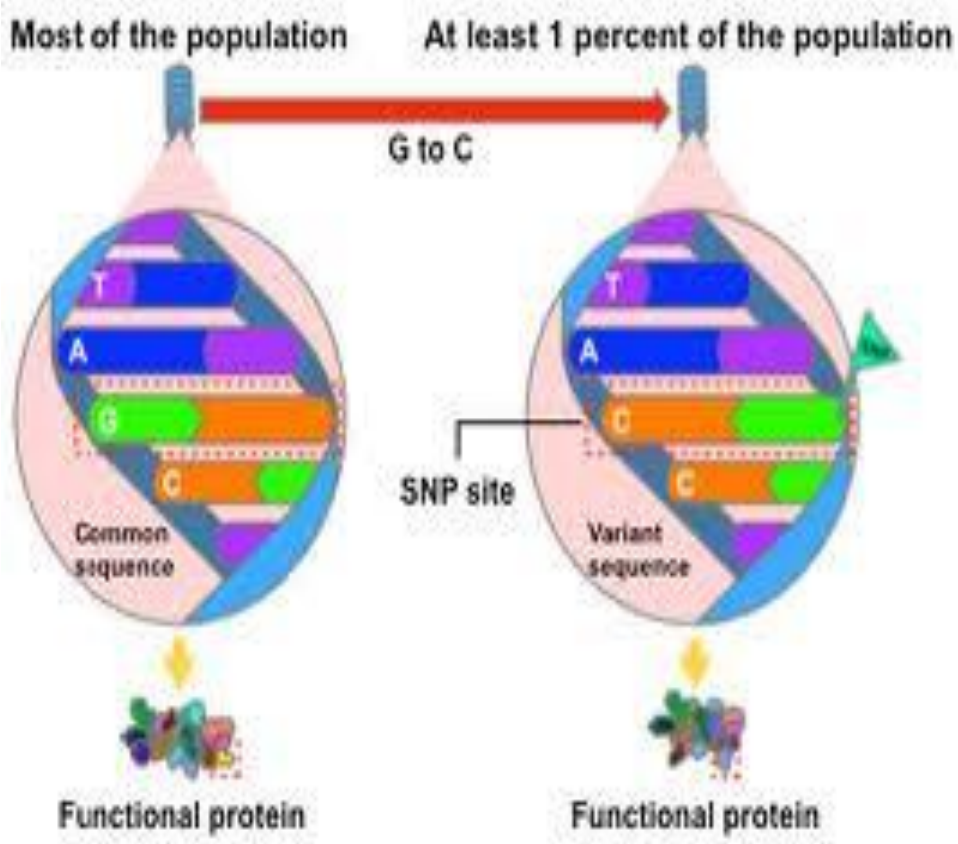




	Thr	Pro	Glu	Glu	$beta^A$ chain
...A C T	C C T	G A G	G A G...	$beta^A$ gene
Codon #	4	5	6	7	
...A C T	C C T	G T G	G A G...	$beta^S$ gene
	Thr	Pro	Val	Glu	$beta^S$ chain

Sickle cell point mutation

glutamic acid to valine substitution codon 6



Polymorphic locus - non-synonymous (missense) mutation if there is a difference in the functionality of the protein produced, the locus is said to have functional polymorphism

nonsense mutation

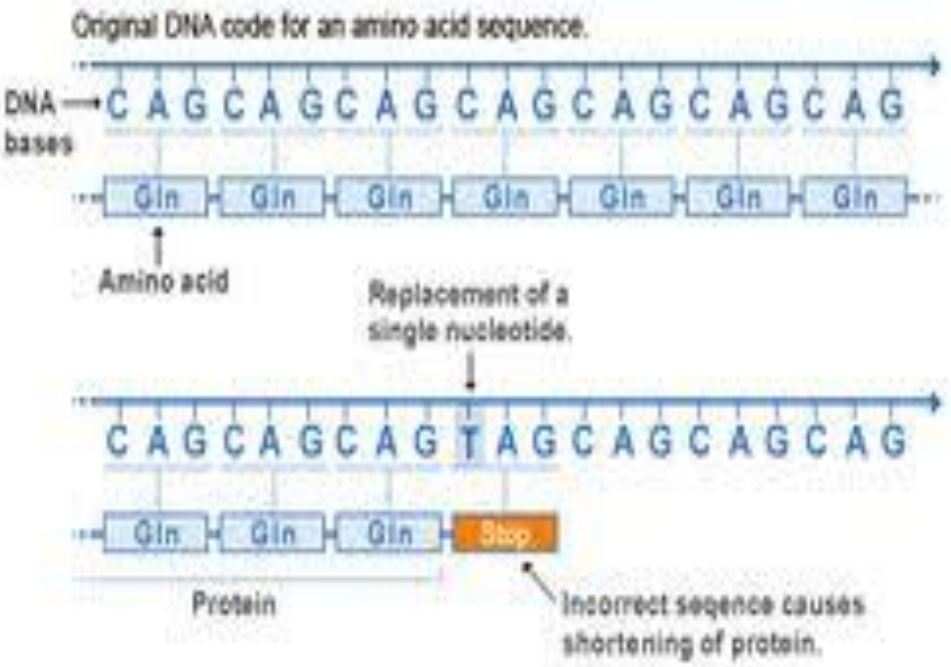
- base-pair substitution results in stop codon and premature ending of polypeptide chain
- result is a functional polymorphism

Duchenne muscular dystrophy

cystic fibrosis

10% of patients have STOP codon instead of amino acid codon in middle of gene

Nonsense mutation



U.S. National Library of Medicine

2. Insertions and deletions

- base-pairs are added or removed from the sequence

frameshift mutation

- triplet reading frame is disrupted if bases are added or deleted (unless multiples of 3 bases are added or deleted)
- result is extensive missense , non-functional product
  - all amino acids after the mutation are altered
  - stop codon may be introduced

wildtype sequence: the big boy saw the new cat eat the hot dog

point deletion: the big oys awt hen ewc ate att heh otd og_

point insertion: the big boy saw tth ene wca tea tth eho tdo g

Molecular Psychiatry (2011) 16, 238–246

www.nature.com/mp

LETTERS TO THE EDITOR

A novel frameshift mutation in *UPF3B* identified in brothers affected with childhood onset schizophrenia and autism spectrum disorders

Molecular Psychiatry (2011) **16,** 238–239; doi:10.1038/mp.2010.59; published online 18 May 2010

Childhood onset schizophrenia (COS) is a rare, severe form of schizophrenia for which definitive genetic causes remain elusive.[1,2] In this study, we report a novel 4-nucleotide deletion in the *UPF3B* gene, predicted to create a truncated protein, transmitted from a healthy mother to two affected brothers: one with comorbid diagnoses of COS, pervasive developmental disorder not otherwise specified and attention deficit hyperactivity disorder (ADHD), and the other with autism and ADHD. This work provides evidence that *UPF3B*, already described as a cause of syndromic and nonsyndromic X-linked mental retardation with or without autism,[3,4] is also involved in COS, autism spectrum disorders and ADHD.

As part of the Synapse to Disease project (http://www.synapse2disease.com/en_projet.html), we sequenced several hundred genes implicated in synapse function and/or structure in 28 probands with COS to identify rare pathogenic disease-causing variants. We amplified and sequenced all coding and intronic flanking regions in *UPF3B* as described previously.[5] PCR primers targeting the 11 exons of *UPF3B* (chrX:118,967,989-118,986,968, NCBI build 37.1; RefSeq NM_080632) were designed using Exon-Primer from the UCSC Genome Browser. We identified a deletion of 4 nucleotides in exon 7 of *UPF3B*, predicted to cause a frameshift (c.683_686delAAGA, p.Q228fsX18) and a truncated *UPF3B* protein in the hemizygous state in a male patient (see Figure 1). This deletion was also identified in his brother diagnosed with autism and transmitted from the unaffected mother. This mutation is located very close to another 4-bp deletion (R225fsX20), recently identified in a family with a diagnosis of FG syndrome and intellectual disability.[4]

Given that the asymptomatic mother was a carrier of the p.Q228fsX18 mutation identified in this study, we determined her X Chromosome Inactivation profile with the Human Androgen Receptor Gene (HUMARA) assay as described previously,[6] using 200 ng of genomic DNA from peripheral blood. Only 24% of mother's blood cells expressed the X chromosome harboring the mutation, indicating that the mother showed a moderately skewed X inactivation pattern with a preferential inactivation (76:24) of the mutated X chromosome allele.

The proband, NSB1442, was the product of an uneventful pregnancy and delivery; developmental milestones were within normal limits. When he first attended school, immediate problems with hyperactivity, immaturity, and impaired social interaction were noted; he was diagnosed with borderline cognitive ability (full scale IQ = 79), ADHD and pervasive developmental disorder not otherwise specified. He was subsequently placed in special education. At the age of 10 years, he began exhibiting paranoid ideation that people and animals were 'out to get him,' and he was hospitalized for one month. At the age of 12 years, he was rehospitalized as a result of inappropriate touching of female peers, aggression towards peers, and threats of violence toward himself and others. Concurrently, he reported auditory hallucinations and delusions that his thoughts and behaviors were being controlled by the voices. He was diagnosed with COS and enrolled in the COS study at the NIMH (National Institute of Mental Health) at the age of 15 years. It is also noteworthy that he received a score of 25 on the ASQ (Autism Screening Questionnaire; score >15 = autism).[7]

The younger brother of the proband, NSB1438, was born with congenital pulmonary stenosis, which precipitated angioplasty attempts at ages 4 and 13 months and a pulmonary valvotomy at 33 months. He had delayed developmental milestones as he did not begin to walk until 16 months and spoke only a few words by 33 months of age. His vocabulary increased by age $3\frac{3}{4}$ years, but he demonstrated significant difficulty with language comprehension, as shown by echolalia and failure to respond to verbal commands/directions with verbal responses. At this time, he also demonstrated significant impulsivity and, after assessment by a special education institution, received diagnoses of ADHD, aphasic language delay, fine motor delay, social delay, and mixed pervasive developmental disorder. He has a full-scale IQ of 87 and was given a formal diagnosis of autism at age 8 years; he scored 31 on the ASQ. Presently at the age of 20 years, he has not experienced any psychotic symptoms.

This is the first report linking a protein truncating mutation in *UPF3B*, a member of the nonsense-mediated mRNA decay complex, as a causative factor in the development of COS, autism spectrum disorders, and ADHD in the same family. The *UPF3B* gene encodes a protein that is part of a postsplicing multiprotein complex involved in both mRNA

triplet repeat mutation

- addition or removal of 3 base-pairs at once will NOT disrupt the reading frame

  delete one codon:

  the big boy the new cat eat the hot dog 1deleted amino acid

  delete across codons:

  the big baw the new cat eat the hot dog 1 amino acid sub for 2

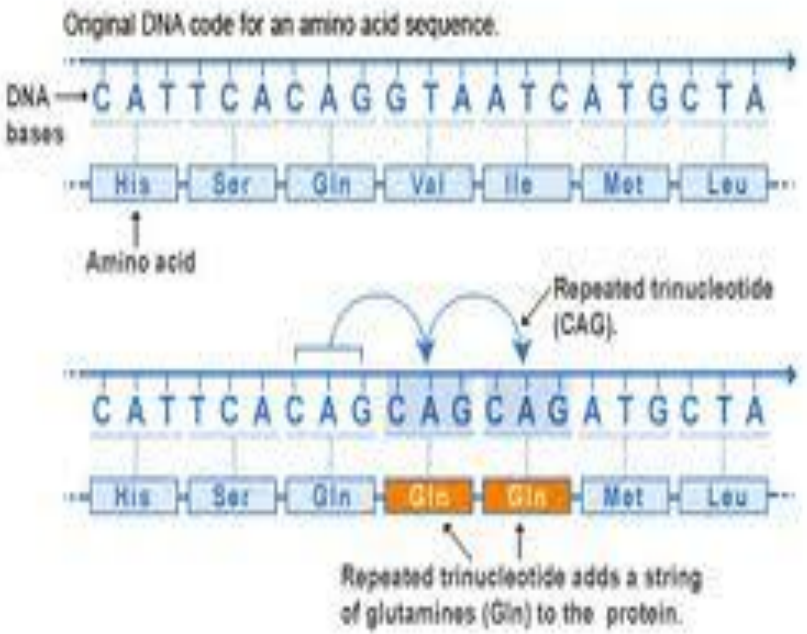
- triplet addition leads to additional amino acids of the same type being added

Huntington mutation CAG repeat polyglutamine

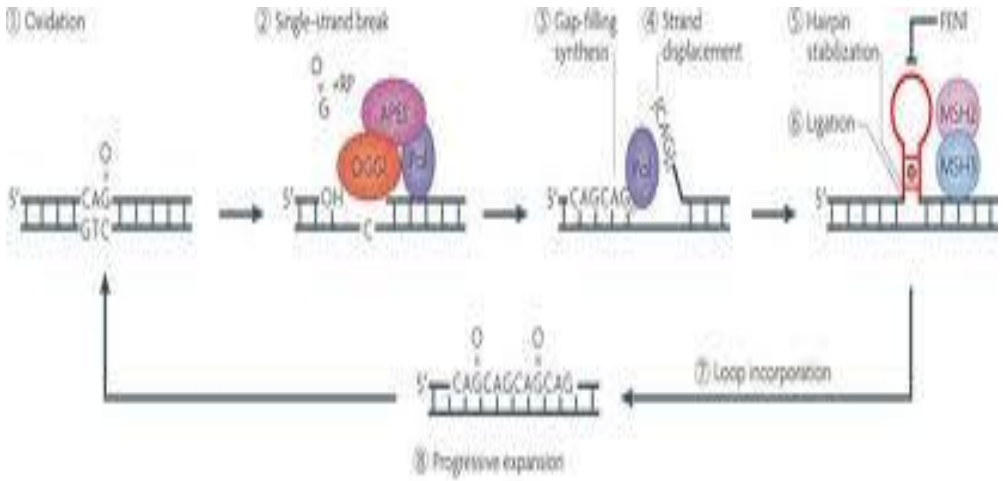
normal= 6-35 repeats

mutation=36-150 repeats

Repeat expansion mutation



U.S. National Library of Medicine



Nature Reviews | Genetics

How are these mutations (polymorphisms) detected?

Fragment-length polymorphisms (and microsatellites) :

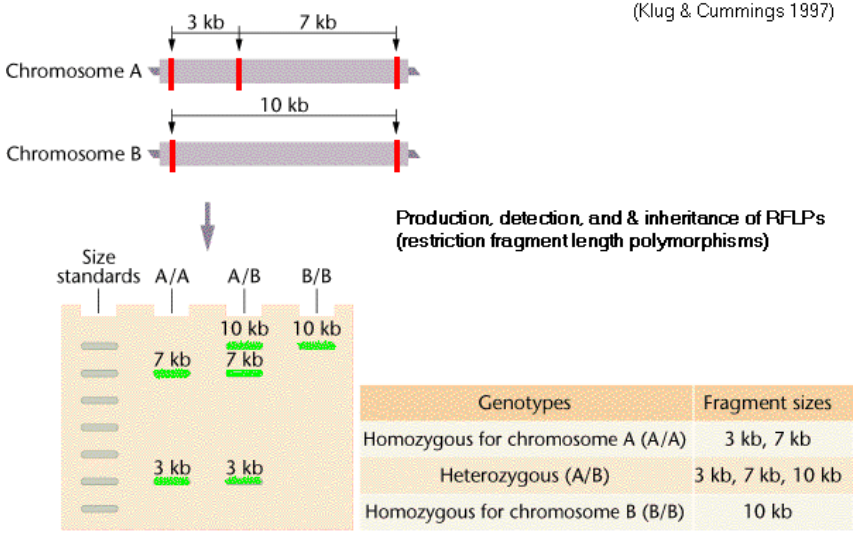
restriction enzymes

- cut DNA at specific points in the sequence

a point mutation may change the restriction point sequence

– DNA will not be cut

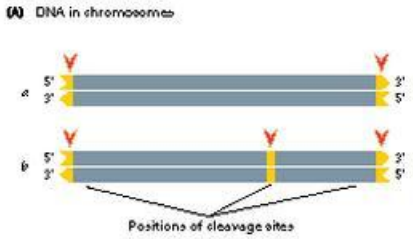
- DNA fragments of different sizes will be detected

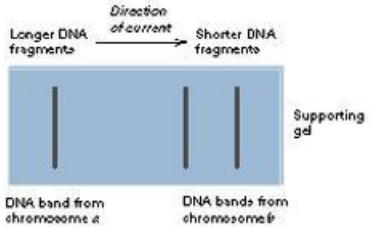


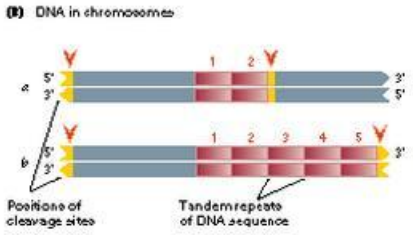
(Klug & Cummings 1997)

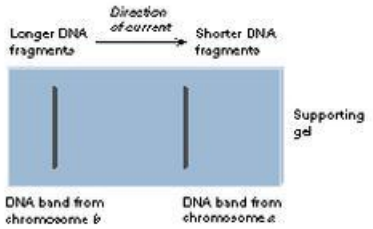
Production, detection, and & inheritance of RFLPs (restriction fragment length polymorphisms)

Genotypes	Fragment sizes
Homozygous for chromosome A (A/A)	3 kb, 7 kb
Heterozygous (A/B)	3 kb, 7 kb, 10 kb
Homozygous for chromosome B (B/B)	10 kb









How are polymorphisms detected? continued

polymerase chain reaction

- amplifies DNA sequence to be studied

http://www.maxanim.com/genetics/PCR/pcr.swf

electrophoresis

- separates DNA fragments for genotyping or identification of markers present

To detect SNPs:

-separate DNA strands, allow to hybridize to single-stranded probe for one or the other allele, fluorescence indicates which probe has been bound and therefore which allele is present

genetic (DNA) marker

- any sequence of known location that varies from person to person and can be genotyped, used to identify regions of DNA associated with variation for a trait

Types of polymorphisms

1. RFLPs - restriction fragment length polymorphisms

2. tandem repeat polymorphisms (microsatellites) - differences in number of copies of a repeated DNA sequence, abundant, highly polymorphic

   simple sequence repeats (SSRs):

   $^{5'}$ACACACACACAC…….$^{3'}$ dinucleotide repeat

   CAGCAGCAGCAGCAGCAG... trinucleotide repeat

   variable number tandem repeats (VNTRs): - repeated unit is +10 nucleotides, easily detected, used in DNA 'fingerprinting'

3. SNPS - single nucleotide polymorphisms, only 2 alleles possible also called SNVs if mutation <1% of population

4. copy number variants duplications of stretches of DNA, microdeletions – deletion of short stretches of DNA

Copy number variants (CNVs)

- elevated burden found in those with schizophrenia, autism, bipolar disorder, intellectual disabilities
- some studies link particular CNVs with the disorder
- most CNVs seem to effect a broad range of disorders
- rarely fully penetrant ie.they act as risk factors
- limited effect
- similar to limited effect of de novo mutations in exons

Autism – de novo exon mutations (Neale et al, Nature, 2012)

- distributed across many genes (confirming highly polygenic nature)
- incompletely penetrant (act as risk alleles)
- exon mutations shown to cause 5-20x increase in risk
- Important but limited effect

Study found:

161 exon point mutations : 101 missense, 50 silent, 10 nonsense,
6 frameshift insertions/deletions

- only the nonsense mutations were at significantly greater frequency than expected
- maternal and paternal age strongly predicted number of de novo mutations
- overall mutation rate only slightly higher than expected
- 1.5×10^{-8} versus expected 1.2×10^{-8} for exon sequences

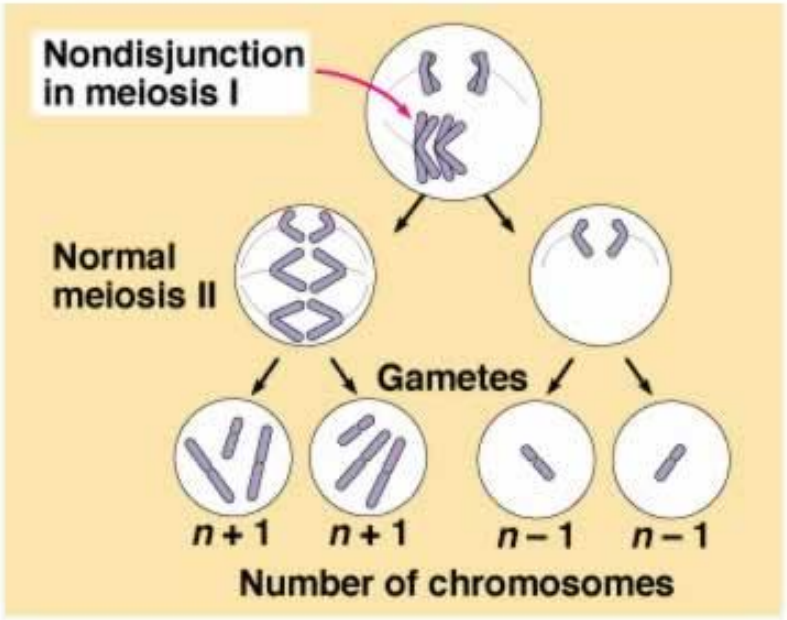
Chromosome mutations

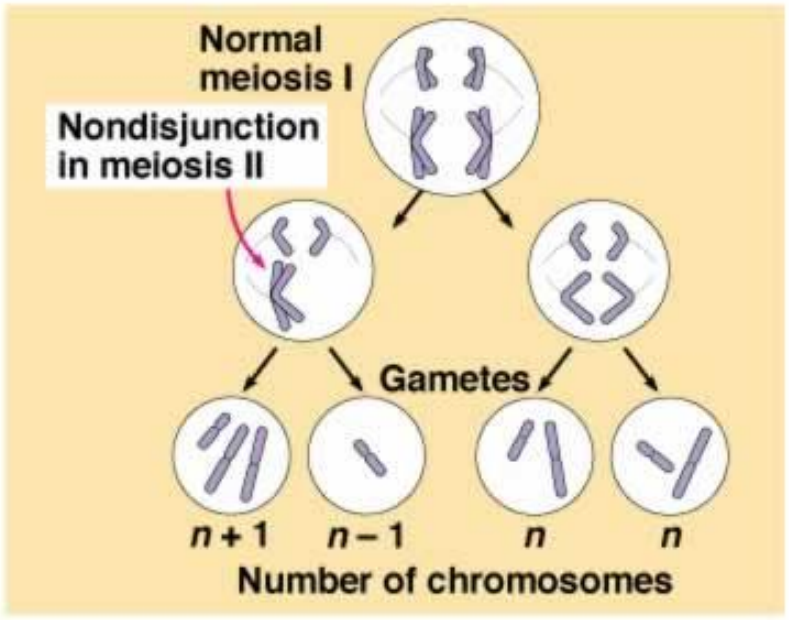
- changes in chromosome number or structure
- more than one gene affected, effects on phenotype more severe

Changes in chromosome number **aneuploidy**

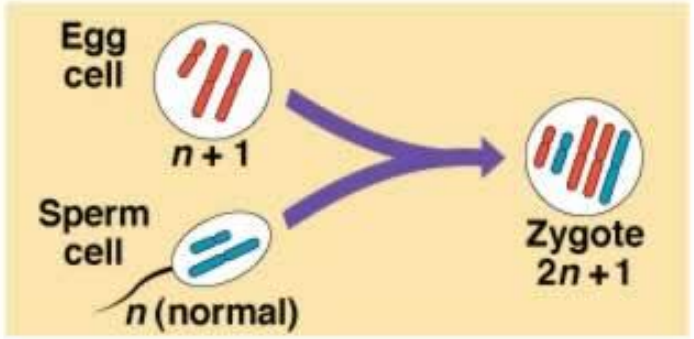
non-disjunction

- process that causes aneuploidy
- failure of homologous chromosomes (or chromatids) to separate during cell division
- unpaired autosomes at meiosis are inactivated so no survival of autosomal monosomies





can happen at mitosis also



Chromosome abnormalities per 100,000 recognized human pregnancies

Chromosome constitution	Number among spontaneously aborted fetuses	Number among live births	
Normal	**7500**	**84,450**	
Trisomy			
13	128	17	
18	223	13	
21	350	113	
Other autosomes	3176	0	
Sex chromosomes			
XYY	4	46	
XXY	4	44	but rising
XO	1350	8	
XXX	21	44	
Polyploid			
Triploid	1275	0	
Tetraploid	450	0	
Other (mosaics etc)	280	49	
Totals	15,000	85,000	

TOTAL with chromosome abnormalities 7500 ie. half of all spontaneous abortions

Human chromosome aneuploidies

- no autosomal monosomies survive
- 3 autosomal trisomies

  all involve small chromsomes with relatively few genes

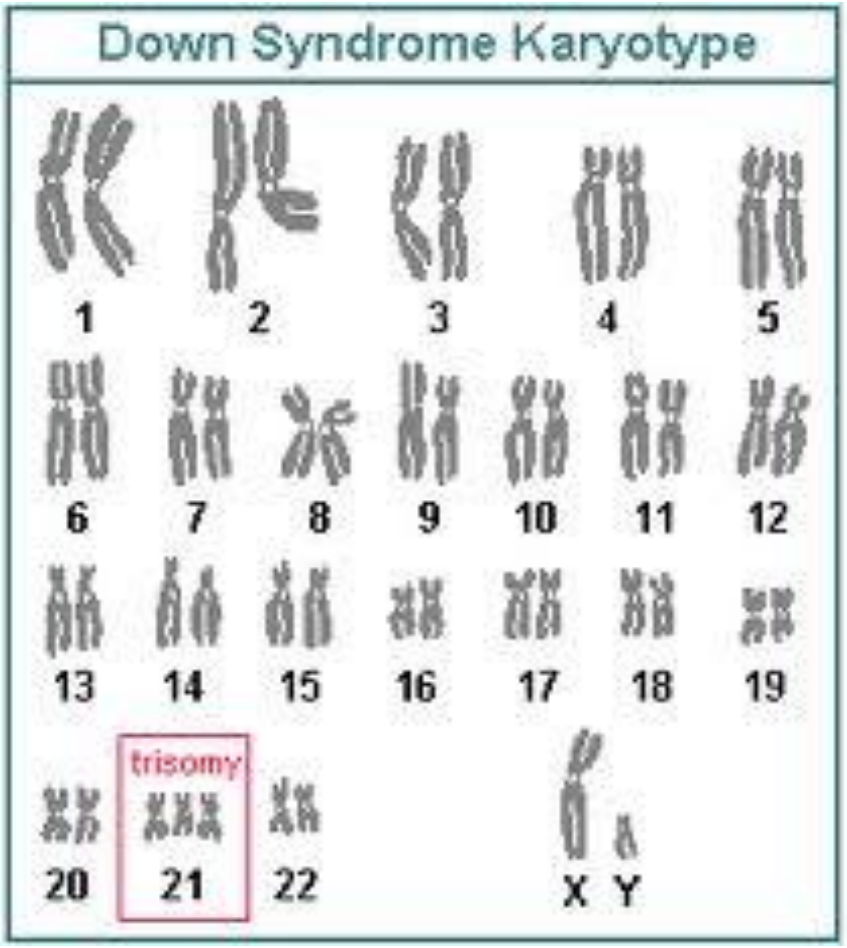
chr 21 374 genes Down syndrome

risk rises with age of mother, from 1 in 3000 at 29 to 1 in 40 at age 45

chr 13 332 genes Patau syndrome

chr 18 243 genes Edward syndrome

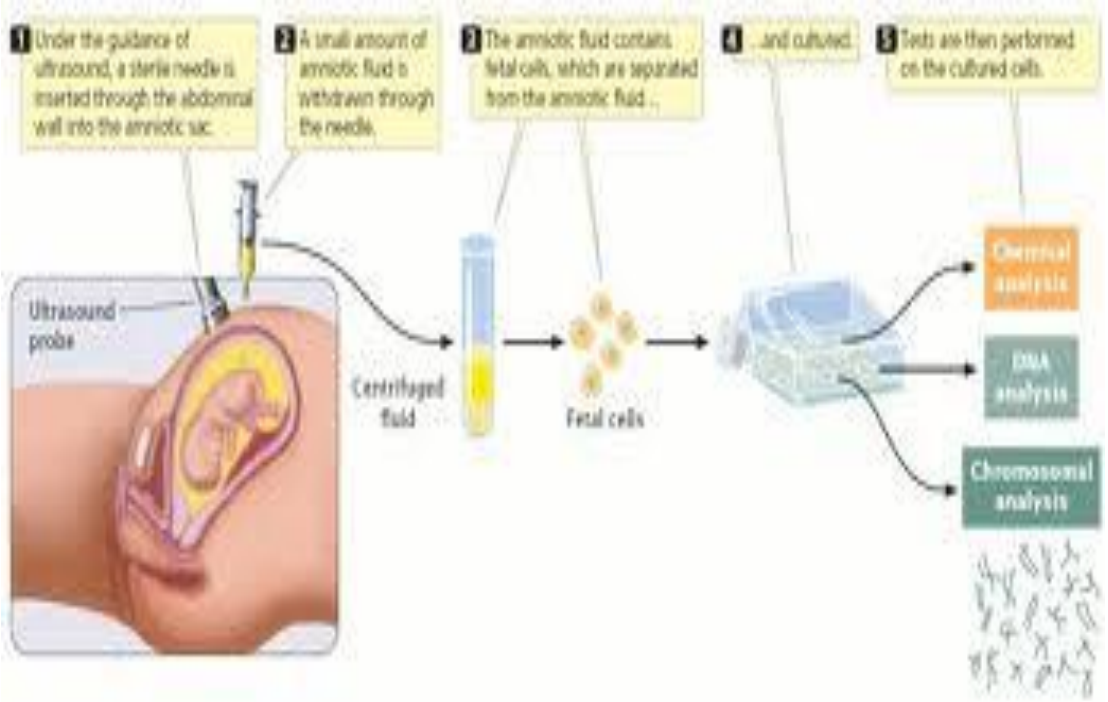
surviving sex chromosome aneuploidies more common



For prenatal diagnosis, how are fetal cells obtained prior to birth?

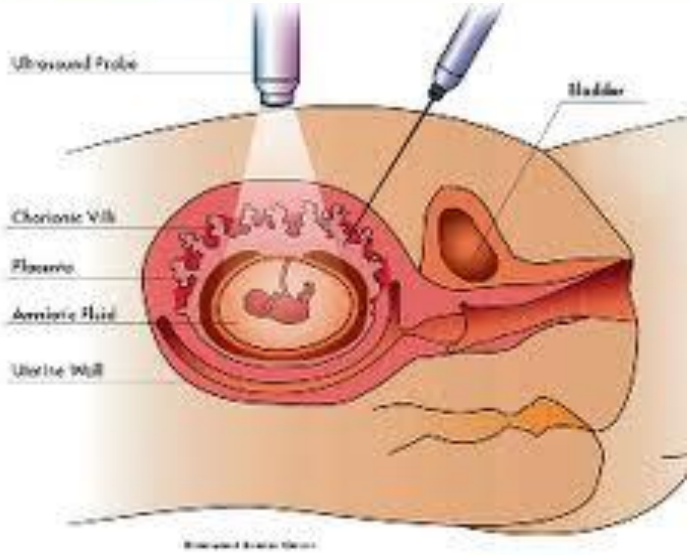
Amniocentesis

14-16 weeks



Chorionic villus sampling

8-10 weeks
but x3 risk of miscarriage above amnio



New non-invasive methods soon to be approved

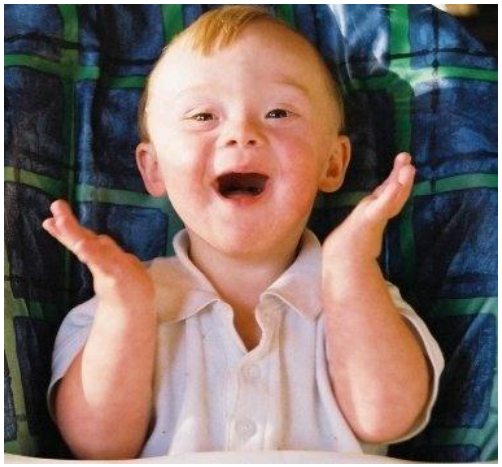
Autosomal trisomies

trisomy 21 Down syndrome

1 in 1000 (average) live births

¼ of all retarded individuals

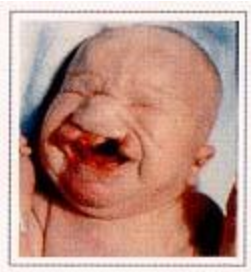
incidence increases with age of Mom

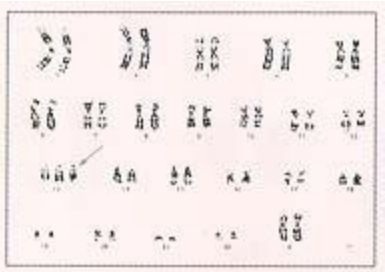


trisomy 13 Patau syndrome

1 in 12,000 live births

fatal, live ~ 3 months

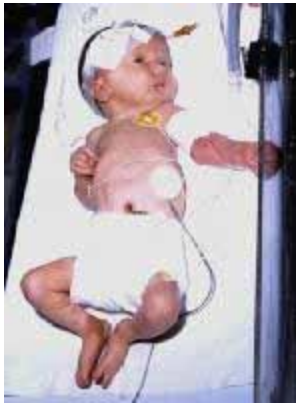




trisomy 18 Edward syndrome

1 in 10,000 live births (1 in 3000, 95% die in utero)

av lifespan = 5-15 days only 5-10% live 1 year



Sex chromosome aneuploidies

- more common, trisomies all around 1 in 1000
- less deleterious since extra X chromosomes are inactivated, Y has few genes

XXY Klinefelter male

1 in 500-1000 live births

almost 2/3 undiagnosed

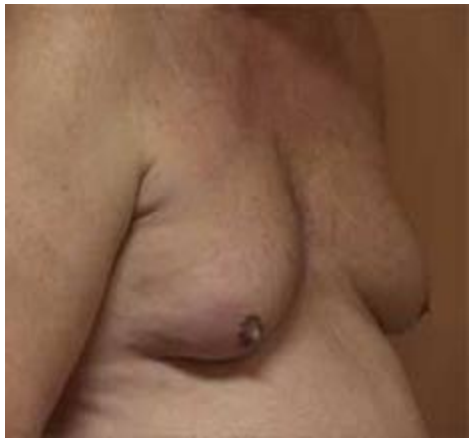
incidence rising

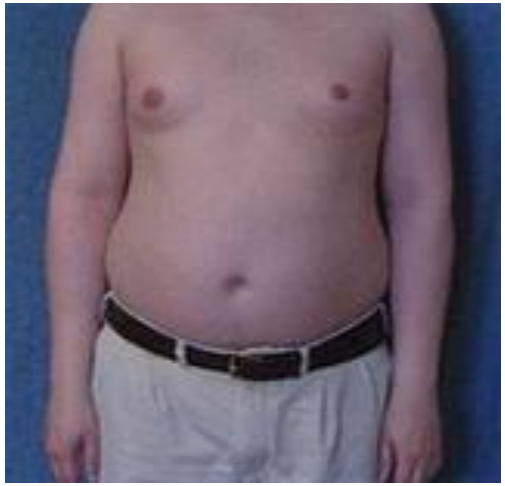
only aneuploidy known to be 50% paternal meiosis I non-disjunction

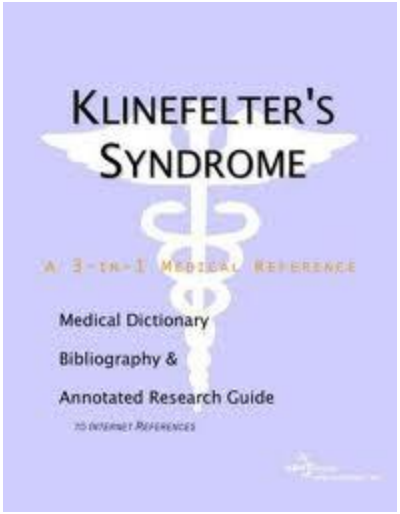
some feminine features unless treated

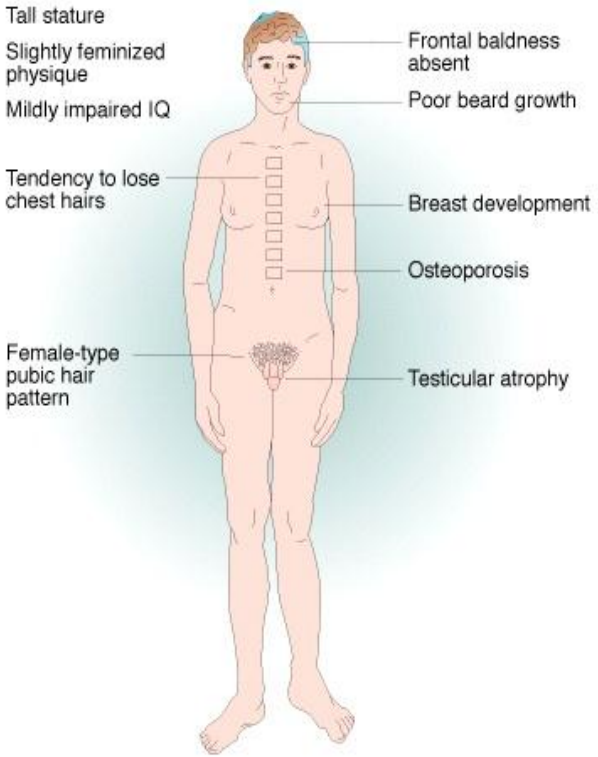
leading cause of male sterility

Untreated

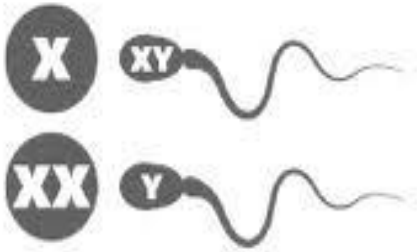




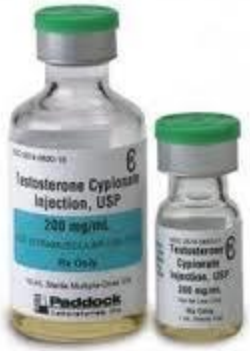

KLINEFELTER'S SYNDROME
A 3-IN-1 MEDICAL REFERENCE
Medical Dictionary
Bibliography &
Annotated Research Guide
TO INTERNET REFERENCES

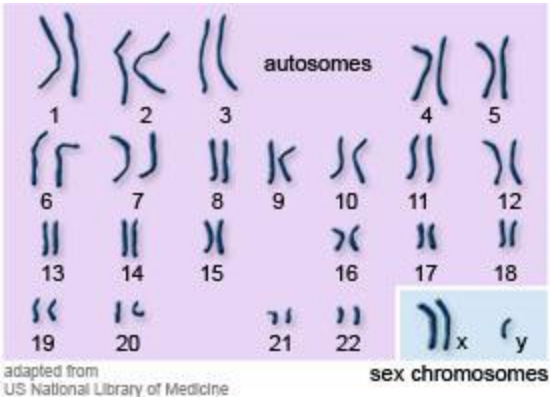

Tall stature
Slightly feminized physique
Mildly impaired IQ
Tendency to lose chest hairs
Female-type pubic hair pattern
Frontal baldness absent
Poor beard growth
Breast development
Osteoporosis
Testicular atrophy

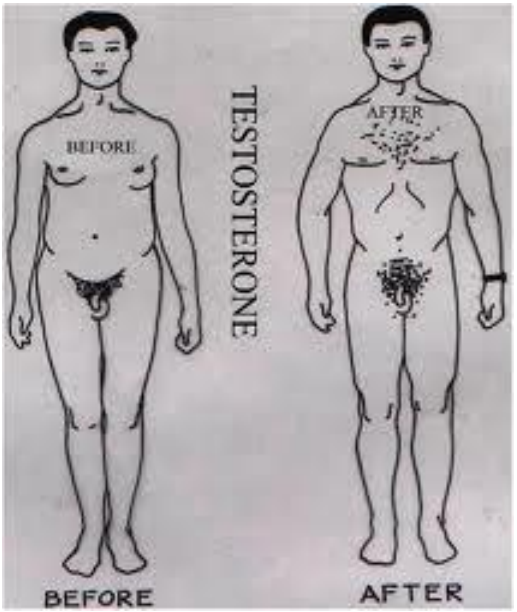
XXY


X
XY
XX
Y

Treated


Testosterone Cypionate Injection, USP
200 mg/mL
Rx Only
Paddock


autosomes
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
x
y
sex chromosomes
adapted from
US National Library of Medicine


BEFORE
AFTER
TESTOSTERONE
BEFORE
AFTER

XXX Triple X female

approx 1 in 1000

normal female, fertile

most undiagnosed

increased risk of learning disabilities

XYY

approx 1 in 1000

normal male, fertile

97% undiagnosed

increased risk of learning disabilities?

XO Turner female

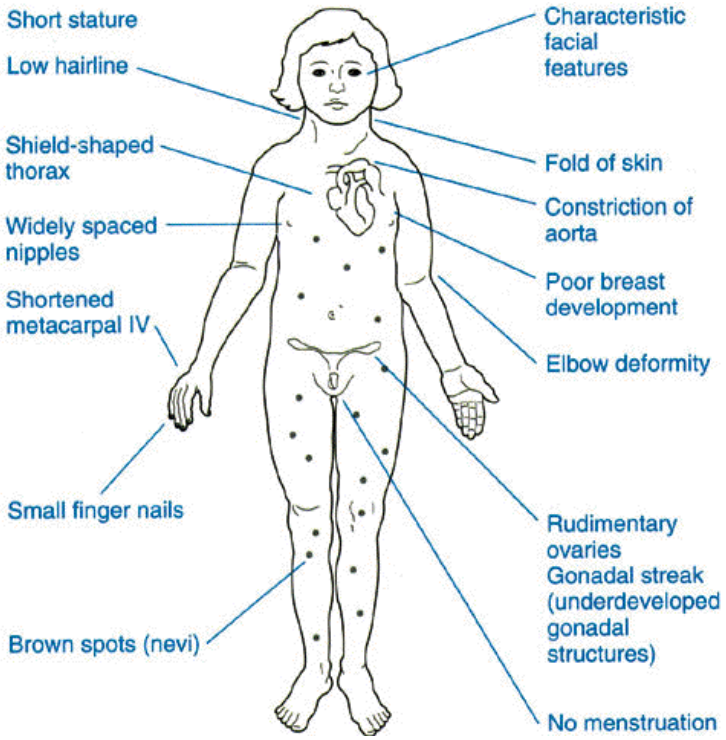
only viable human monosomy

1 in 3000 live births

sterile

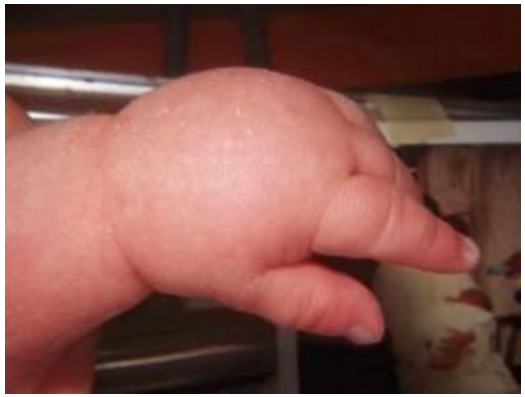
no secondary sex characteristics

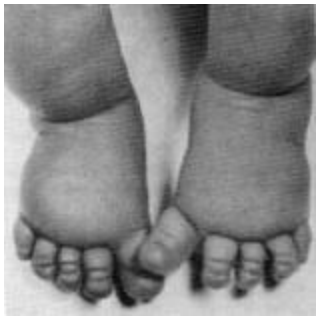
untreated



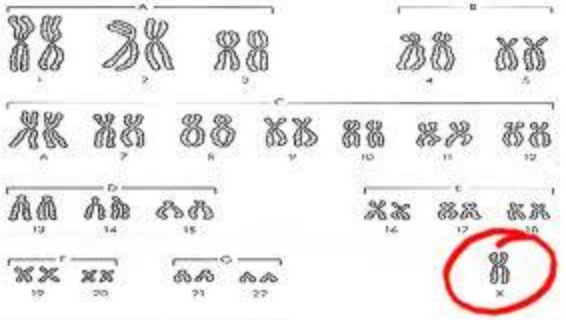
Turner Syndrome

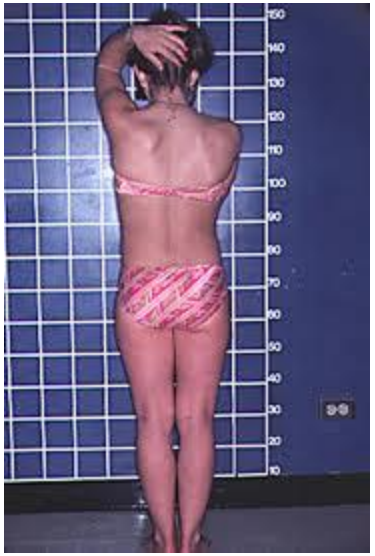
At birth





Karyotype





Treatment

NutropinAq®
[somatropin, recombinant DNA origin, *Escherichia coli*]

Katie with some friends who also have Turner Syndrome