Aberrant Folding of a Mutant Stat5b Causes Growth Hormone Insensitivity and Proteasomal Dysfunction*

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A predicted alanine to proline substitution in Stat5b that results in profound short stature, growth hormone insensitivity, and immunodeficiency represents the first natural mutation of this transcription factor in a human. To understand the mechanisms responsible for these pathophysiological abnormalities, we have studied the biochemical and biophysical properties of the mutant Stat5b molecule. In a cellular reconstitution model growth hormone robustly stimulated tyrosine phosphorylation and transcriptional activity of wild-type Stat5b while Stat5bA630P was minimally modified and did not promote reporter gene expression. Steady state levels of Stat5bWT were 3-fold higher than Stat5bA630P in cell extracts prepared with nonionic detergents. Although initial rates of biosynthesis of both proteins were similar, pulse-chase experiments established that the apparent half-life of newly synthesized soluble Stat5bA630P was 15% of Stat5bWT (3.5 h versus >24 h). Stat5bA630P accumulated in cells primarily in cytoplasmic inclusion bodies. Structural analysis of the isolated SH2 domain containing the A630P mutation showed that it resembled the wild-type SH2 segment but that it exhibited reduced thermodynamic stability and slower folding kinetics, displayed an increased hydrophobic surface, and was prone to aggregation in solution. Our results are compatible with a model in which Stat5bA630P is an inactive transcription factor by virtue of its aberrant folding and diminished solubility triggered by a misfolded SH2 domain. The potential for aggregation and formation of cytoplasmic inclusions raises the possibility that Stat5bA630P could produce additional defects through inhibition of proteasome function.

Sequence-specific transcription factors are modular proteins containing distinct domains that mediate their actions, including the ability to move among different subcellular compartments, to bind to DNA in chromatin in the nucleus, and to interact with transcriptional co-activators, co-repressors, and other regulatory molecules. The Stat family consists of seven related proteins (Stats 1, 2, 3, 4, 5a, 5b, and 6) that are responsible for many of the transcriptional effects of cytokines, growth factors, and hormones (2, 3). Stat proteins are found in latent form in the cytoplasm of unstimulated cells. They are activated upon ligand binding to its receptor by a series of steps consisting of receptor-initiated tyrosine phosphorylation, dimerization, transport into the nucleus, binding to DNA response elements on target genes, and recruitment of a complex of co-activator proteins that stimulate transcription (2, 3).

Growth hormone (GH) plays a central role in regulating somatic growth and intermediary metabolism in many vertebrate species, including humans (4). Upon binding to its transmembrane receptor, GH triggers the activation of the receptor-associated tyrosine-protein kinase Jak2, which in addition to recruiting a variety of other signaling molecules leads to the activation of Stats 1, 3, 5a, and 5b (4, 5). Many of the growth promoting actions of GH are mediated by the peptide growth factor, IGF-I (6–8), and IGF gene expression is under the control of GH (9). Recent studies have demonstrated that GH stimulates IGF-I gene transcription through the mediation of Stat5b (10), and results in experimental animals have shown that the absence of Stat5b is associated with diminished post-natal growth (11, 12).

Decreased activity through the GH-IGF-I axis causes growth defects in humans that may lead to short stature (13, 14). Several genetic and acquired abnormalities have been shown to inhibit GH gene expression or biosynthesis (13), and mutations in the GH receptor have been described that impair its synthesis or biological actions (15, 16). Individuals also have been reported with growth deficiency and inactivating mutations in the genes for IGF-I and its receptor (17–19). The recent description of a young woman with markedly impaired growth and a homozygous point mutation in the Stat5b gene predicted to change amino acid alanine 630 to proline within the SH2 domain of the protein (20) prompted us to investigate the biochemical consequences of this inherited defect. Here we demonstrate that Stat5bA630P is an inactive transcription factor. In cells, including those derived from the patient, Stat5bA630P accumulates primarily as stable protein aggregates in cytoplasmic inclusion bodies. The A630P mutation also predisposes the isolated SH2 domain to aggregate in solution under conditions in which the wild-type domain is a monomer. Our observations demonstrate that this individual has a protein folding disorder where the aberrantly folded SH2 domain of the mutant Stat5b molecule triggers primary transcription factor deficiency and protein aggregation.

EXPERIMENTAL PROCEDURES

Recombinant Plasmids and Adenoviruses—An expression plasmid in pcDNA3 and an adenovirus encoding NH2-terminal FLAG-tagged rat Stat5bWT have been described previously (10), as have an expression plasmid for the mouse GH receptor and for FLAG-tagged rat Stat5bY699F and reporter plasmids HS7-TK-Luc and HS7-IGF P1-Luc (21). Codon 630 of FLAG-Stat5bWT was modified by site-directed mutagenesis (Stratagene, La Jolla, CA) to produce FLAG-Stat5bA630P in pcDNA3, and an adenovirus encoding this protein under the control of a tetracycline-repressible promoter was prepared and purified as described (10). DNA encoding the SH2 domains of Stat5bWT and
Stat5b<sub>AG30P</sub> (from codon Asp<sup>591</sup> to Glu<sup>689</sup>) was generated by PCR and cloned into pET-15b (Novagen, San Diego, CA) using NdeI and XhoI restriction sites so that a hexahistidine tag was added to the NH<sub>2</sub> terminus. The coding regions of all recombinant plasmids were verified by DNA sequencing.

**Cell Culture and Transient Transfections**—All of the cells were maintained in Dulbecco’s modified Eagle’s medium (Mediatech-Cellogro, Herndon, VA) supplemented with 10% heat-inactivated fetal bovine serum at 37 °C in humidified air with 5% CO<sub>2</sub>. Preparation of primary dermal fibroblasts from the patient homozygous for the A630P mutation in Stat5b and from a 30-year old female of normal height has been described (20). All transient transfections were performed with TransIT-LT1 (Mirus, Madison, WI) and a protocol from the supplier.

**Reporter Gene Assays**—Cos-7 cells (ATCC CRL-1651) were transfected in 6-well tissue culture dishes with expression plasmids for mouse GH receptor (100 ng), Stat5b<sub>WT</sub> or Stat5b<sub>AG30P</sub> (100 ng), and the reporter plasmids H7-TK-Luc or H7-IGF P1-Luc (250 ng). After 24 h, the cells were transfected with 50% mixture of methanol and acetone for 2 min before blocking in 0.25% normal goat serum for >1 h at 20 °C. After the addition of the FLAG M2 monoclonal antibody at 1:2000 dilution in blocking buffer overnight followed by a washing step and incubation in goat anti-mouse IgG<sub>1</sub>–Alexa 488 at 1:1000 dilution in blocking buffer for 2 h, the images were captured with a Roper Scientific Cool Snap FX CCD camera attached to a Nikon Eclipse Ti-E fluorescence microscope using IP Labs 3.5 software (Scanalytics, Rockville, MD).

**Purification of SH2 Domain**—Escherichia coli strain BL21 cells transformed with pET15b-Stat5b<sub>WT</sub>, or pET15b-Stat5b<sub>AG30P</sub> SH2 domains were grown with shaking at 37 °C in LB medium containing ampicillin (200 µg/ml) to an A<sub>600nm</sub> of 0.6–1.0 and induced with 1 mM isopropyl-β-D-thiogalactopyranoside for 4 h. Purification of the His-tagged SH2 domains from inclusion bodies was as previously described (22). Upon elution from the nickel-nitrilotriacetic acid-agarose column, the fractions were dialyzed against 100 volumes of refolding buffer (20 mM Na<sub>2</sub>HPO<sub>4</sub>/KH<sub>2</sub>PO<sub>4</sub>, pH 7.5, 150 mM NaCl, 0.5 mM EDTA, 1 mM β-mercaptoethanol) with two buffer changes. Purification and yield were monitored by SDS-PAGE and staining with GelCode blue reagent (Pierce). For gel filtration chromatography and binding studies with 1-anilino-8-naphthalenesulfonic acid (ANS), purified samples were used immediately after refolding.

**Circular Dichroism Spectroscopy**—CD measurements were performed on an AVIV model 215 CD spectrometer maintained at 4 °C. The spectra were measured from 260 to 200 nm at 0.5-nm intervals. The protein concentration was 18.6 µM, and a path length of 0.1 cm was used. The measurements were averaged over a 3-s time interval at each wavelength. The profiles depicted in Fig. 4 are averages of three independent experiments.

Equilibrium folding and unfolding were monitored by CD absorbance at 222 nm upon increasing guanidine HCl concentrations from 0 to 6 M at 0.25 M intervals to obtain free energy differences for each transition as described previously (23). The unfolding transitions best represented two states, native (N) and unfolded (U), as follows.

\[
K_{NU} = \frac{N}{U}
\]

The observed ellipticity, \(A_{obs}(c)\), at any concentration of the denaturant is given by the sum of the contributions from the two states as follows,

\[
A_{obs}(c) = \frac{A_N + A_U\exp \left[-\left(\Delta G_{NU}^{\text{H2O}} - m_{NU}(c)/RT\right)\right]}{1 + \exp \left[-\left(\Delta G_{NU}^{\text{H2O}} - m_{NU}(c)/RT\right)\right]}
\]

where \(A_N\) and \(A_U\) are the ellipticity values of the pure N and U states, respectively, \(\Delta G_{NU}^{\text{H2O}}\) is \(\Delta G_{NU}\) at 0 M guanidine HCl, and \(m_{NU}c\) represents
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the dependence of the respective free energy changes on \( c \). The data were fitted using Prism Graphpad.

Folding Kinetics—The change in fluorescence upon folding-unfolding was used to monitor kinetics. Stopped flow kinetics were measured on a Hi-Tech SF61-DX2 fluorometer using \( \lambda_{\text{excitation}} \), of a 295- and 310-nm band pass filter. For these analyses, unfolded proteins (1–4 \( \mu \)M) in 6 M guanidine HCl were rapidly diluted (1:10 ratio) into renaturation buffer. The data were fitted to single and double exponential equations (GraphPad-Prism) to determine folding rate constants. All of the experiments were performed at 23°C, and the results shown represent the averages of three to six independent replicates.

Fluorescence Spectroscopy—The fluorescence measurements were performed on a Photon Technology International spectrometer with a model 810 photomultiplier detection system at 4°C. The protein samples (2.5 \( \mu \)M) were immediately incubated with ANS (50 \( \mu \)M) for 30 min upon completion of refolding. The proteins were excited at 390 nm, and the emission scan was recorded from 400 to 600 nm. The excitation and emission bandwidths were maintained at 10 nm throughout the experiments. For the intrinsic fluorescence experiments, the protein samples (5 \( \mu \)M) were excited at 295 nm, and the emission scans were recorded from 300 to 400 nm.

Gel Filtration Chromatography—Soluble protein (250 ml at 1 mg/ml) was applied to a Superdex S200 HR10/30 column (Amersham Biosciences) previously equilibrated with 20 mM Na2HPO4/KH2PO4, pH 7.5, 150 mM NaCl, 0.5 mM EDTA, 1 mM \( \beta \)-mercaptoethanol. The proteins were eluted at 20°C using a flow rate of 0.5 ml/min. The elution was monitored at 280 nm using a Beckman Gold 166 UV-visible detector.

Molecular Modeling—Modeling was performed using Swiss Model, a program that predicts structures reliably (root mean square deviation < 2 Å) for sequences with at least 50–60% identity, using coordinates from the crystal structure of human Stat1 (Swiss Protein Database 1YVL) as the template (24).

RESULTS

Absent Transcriptional Activity and Diminished Tyrosine Phosphorylation of Stat5b\( ^{A630P} \) after GH Treatment—The overall domain structure of Stat5b is pictured in Fig. 1A. To study the consequences of the A630P substitution within the SH2 domain of the protein, we engineered the mutation into FLAG epitope-tagged rat Stat5b, which is 96% identical to the human protein (25), and generated recombinant expression plasmids and adenoviruses. In initial experiments we addressed the potential transcriptional properties of the mutant protein and the effects of acute GH treatment on its tyrosine phosphorylation after transient transfection with the GH receptor into Cos-7 cells. As seen in Fig. 1B, in cells expressing Stat5b\( ^{A630P} \), GH had no stimulatory effect on the activity of reporter genes containing a GH-responsive element from the rat IGF-I gene (21). In contrast, hormone treatment led to a 4- or 7-fold rise in reporter gene expression in cells transfected with Stat5b\( ^{WT} \).

The earliest event in GH-stimulated activation of Stat5b is recruitment of the protein to the tyrosine-phosphorylated cytoplasmic tail of the GH receptor, followed by phosphorylation of tyrosine 699 of Stat5b (\( \text{Y} \)) and robust tyrosine phosphorylation of Stat5bWT but had a minimal rise in reporter gene expression in cells transfected with Stat5b\( ^{WT} \) or Stat5b\( ^{A630P} \). As seen in Fig. 1C, GH caused rapid and robust tyrosine phosphorylation of Stat5b\( ^{WT} \) but had a minimal effect on Stat5b\( ^{A630P} \), because levels of its tyrosine phosphorylation were barely above those of the nonphosphorylated Stat5b\( ^{Y699F} \) mutant. Thus, based on these results, Stat5b\( ^{A630P} \) is transcriptionally inert secondary to its inability to be activated by GH.

Diminished Expression of Stat5b\( ^{A630P} \) Is Secondary to Its Decreased Stability—Analysis of steady state protein levels in transfected Cos-7 cells revealed that the amount of Stat5b\( ^{A630P} \) was only ~30–35% of Stat5b\( ^{WT} \) (Fig. 24). Treatment with GH had no effect on the accumulation of either protein (data not shown). Assessment of the biosynthetic rates of each transcription factor after infection of Cos-7 cells with recombinant adenoviruses showed that each protein could be detected within 2 h of infection, but that Stat5b\( ^{A630P} \) reached constant levels sooner than Stat5b\( ^{WT} \) (8 versus <24 h), and that its apparent concentration at steady state was reduced by ~70% compared with Stat5b\( ^{WT} \) (Fig. 2B). Evaluation of protein half-life by pulse-chase assays demonstrated that Stat5b\( ^{A630P} \) was a fairly long-lived protein, with a \( T_{1/2} \) > 24 h, whereas Stat5b\( ^{A630P} \) appeared to be short-lived, with a \( T_{1/2} \) of ~3.5 h (Fig. 2C and data not shown). Thus, at least in transfected cells, in addition to being transcriptionally silent secondary to reduced activation by GH, Stat5b\( ^{A630P} \) appears to turn over at least seven times faster than Stat5b\( ^{WT} \).

Stat5b\( ^{A630P} \) Accumulates in Cells in Insoluble Protein Aggregates That Interfere with Proteasomal Function—We next addressed the mechanism of apparently increased turnover of Stat5b\( ^{A630P} \) by assessing the potential role of lysosomal or proteasomal degradation. Neither steady state protein levels nor the \( T_{1/2} \) of either Stat5b\( ^{WT} \) or Stat5b\( ^{A630P} \) were altered by incubation of cells with a variety of lysosomal and proteasomal inhibitors including chloroquine, MG132, and N-acetyl-Leu-Leu-Nle-CHO (where Nle is norleucine; data not shown). Some disease-associated proteins when mutated, including the cystic fibrosis transmembrane conductance regulator (26), rhodopsin (27, 28), and...
huntingtin (29), have been shown to be misfolded, and at least when overexpressed, form insoluble protein aggregates in cells (26). To determine whether Stat5bA630P is prone to aggregation, we looked for accumulation of the protein in the "insoluble" fraction after extraction of transfected cells with buffer containing primarily nonionic detergents. As seen in Fig. 3A and in contrast to Stat5bWT and tubulin, which were found exclusively in the soluble fraction, over 80% of Stat5bA630P was located in the insoluble portion and could be solubilized fully only with high concentrations of ionic detergents. Analogous results were observed when transfected Cos-7 cells were evaluated by immunocytochemistry. In the absence of GH, Stat5bWT was found throughout the cytoplasm in a diffuse and uniform pattern, whereas Stat5bA630P was concentrated in multiple discrete cytoplasmic inclusions (Fig. 3B), which have been termed "aggresomes" when detected previously in cells expressing proteins prone to misfolding (26).

To determine whether aggregates of Stat5bA630P occurred in an endogenous setting, we examined skin fibroblasts derived from the index patient. As pictured in Fig. 3C, unlike cells from an individual expressing Stat5bWT, where the protein was found only in the soluble fraction, in the fibroblasts of the patient ~90% of Stat5bA630P was located in the insoluble portion. By contrast, in cells from both individuals highly related Stat5a (91% identical to Stat5b (25)) partitioned to the soluble fraction. We were unable to observe inclusion bodies in the cells of the patient by immunocytochemistry (data not shown), a finding that we interpret as secondary to low levels of expression of Stat5b in fibroblasts. Based on our results, the accumulation of Stat5bA630P in an insoluble protein complex is an inherent property of the mutant protein and is not just induced by its overexpression in Cos-7 cells.

Several studies have suggested that proteins that aggregate may disrupt proteasome function, leading to alterations in metabolism of short-lived cellular proteins (30, 31). To determine whether Stat5bA630P could cause gain-of-function abnormalities secondary to diminished proteasomal activity, we examined in Cos-7 cells steady state levels of co-expressed MyoD, a muscle-specific transcription factor with a $T_{1/2}$ of ~30 min (32). As pictured in Fig. 3D, Stat5bWT had no effect on levels of MyoD when co-expressed with Stat5bA630P and fractionated by solubility in radioimmune precipitation assay (RIPA) buffer. S, soluble; I, insoluble. B, immunocytochemistry of transiently transfected Cos-7 cells for Stat5b using FLAG M2 antibody (green). The nuclei are stained with Hoechst dye (blue). C, results of pulse-chase experiments after transient transfection of Cos-7 cells with expression plasmids for Stat5bWT or Stat5bA630P. The relative protein levels are indicated.
MyoD, whereas co-expression with Stat5b\textsuperscript{A630P} led to a >10-fold increase in its abundance, similar to that seen after incubation with the proteasome inhibitor N-acetyl-Leu-Leu-Nle-CHO. Based on these results, it is possible that some of the pathologial changes observed in the individual with the homozygous Stat5b\textsuperscript{A630P} mutation are secondary to alterations in levels of selected proteins because of diminished proteasome function.

**Misfolding of the Isolated SH2 Domain of Stat5b\textsuperscript{A630P}**—To understand how the A630P mutation might perturb the structure and function of the SH2 domain, we cloned, expressed, and characterized the isolated SH2 domains of wild-type and mutant human Stat5b. Both proteins were purified from an *E. coli* expression system to >95% purity (data not shown) and refolded as described under “Experimental Procedures.” We first analyzed the effect of the A630P substitution on the secondary and tertiary structure of the Stat5b\textsuperscript{A630P} mutation. To determine whether the A630P substitution altered solvent-exposed hydrophobic surfaces within the SH2 domain, we analyzed binding of the dye ANS to folded wild-type and mutant peptides. ANS exhibits an increase in its fluorescence upon binding to exposed hydrophobic surfaces, with a shift in \(\lambda_{\text{max}}\) toward a lower wavelength. As depicted in Fig. 4E, the extrinsic fluorescence intensity of the A630P SH2 domain is greater than that observed with wild-type peptide and is consistent with an enhanced hydrophobic surface area. Based on these results, it is possible that some of the pathological changes observed in the individual with the homozygous Stat5b\textsuperscript{A630P} mutation are secondary to alterations in levels of selected proteins because of diminished proteasome function.

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*Figure 4.* The isolated SH2 domain of Stat5b\textsuperscript{A630P} is more energetically unstable than the SH2 domain of Stat5b\textsuperscript{WT} and undergoes aggregation in solution. **A,** circular dichroism spectra of guanidine HCl denatured (triangles) and folded (circles) SH2 domains. Folded WT-SH2 domain (filled circle) displays greater negative ellipticity when compared with the A630P SH2 domains (open circle). **B,** intrinsic tryptophan fluorescence spectra. Wild-type and A630P SH2 domains display similar fluorescence intensity with a 1-nm shift in the fluorescence maximum. **C,** denaturant induced folding-unfolding equilibrium of wild-type (filled circles) and A630P SH2 domains (open circles). Structural transitions were monitored using changes in CD absorbance at 222 nm. **D,** kinetic assessment of folding-unfolding transition. Stopped flow kinetics were measured upon dilution of unfolded wild-type (black line) and A630P (gray line) SH2 domains into renaturation buffer. **E,** ANS induced extrinsic fluorescence spectra. The fluorescence maximum of ANS shifts from 510 to 476 nm for both wild-type (filled circles) and A630P (open circles) SH2 domains. The fluorescence intensity for the mutant protein is greater than that for the wild-type protein and is consistent with an increase in solvent-exposed hydrophobic surface. **F,** size exclusion chromatography performed on a Superdex S200 HR 10/30 column, as outlined under “Experimental Procedures.” Protein elution was monitored at 280 nm. The arrows indicate standard markers and their corresponding molecular masses.
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FIGURE 5. The A630P mutation maps to a conserved β2 strand. A, sequence and structural conservation among SH2 domains of human Stat proteins. The site of the mutation in Stat5b is highlighted in green in all seven Stats. The composite secondary structure is displayed below the sequence alignment. B, ribbon diagram of the SH2 domain of Stat5b*A630P. The structure was based on a homology model obtained using the Swiss Model program and the coordinates from the crystal structure of human Stat1 (Swiss Protein Database 1YVL) as the template (23). The mutant proline residue is highlighted in green.

ated by evidence of reduced negative ellipticity by CD and by the lowered thermodynamic stability. When considered in conjunction with evidence of an increased exposed hydrophobic surface and a larger Stokes radius, these results establish that the A630P SH2 domain may adopt a classical molten globule-like state. Because molten globule-like states are well established intermediate protein conformations that are prone to aggregation (33), the aberrant folding of the mutant SH2 domain provides a biophysical basis for the aggregation of Stat5b*A630P observed in cells.

**DISCUSSION**

Recent observations support the idea that Stat5b plays a pivotal role in the GH-IGF-I growth pathway in humans and other mammalian species. A targeted knockout of Stat5b in mice results in diminished post-natal growth (11, 12), and biochemical studies have established that GH requires Stat5b to mediate hormone-induced IGF-I gene transcription (10). Two humans with profound growth deficiency who harbor homozygous Stat5b mutations have been identified (20, 34). One individual has a single nucleotide insertion in exon 10 of the Stat5b gene that results in a frameshift mutation and undetectable protein expression (34). The other has a mis-sense mutation that changes alanine 630 to proline within the SH2 domain of the protein (20). The studies described here show that Stat5b*A630P is an inactive transcription factor. By virtue of its aberrant folding and aggregation triggered by an abnormally structured SH2 domain, the majority of mutant Stat5b*A630P both in the fibroblasts of the patient and when overexpressed in Cos-7 cells is not soluble, and the remainder is only minimally activated upon GH stimulation, as evidenced by the near lack of GH-induced tyrosine phosphorylation, and the absence of transcriptional stimulation of reporter genes.

The amino acid sequences of all seven Stat proteins display substantial conservation in the structured regions within their SH2 domains, with differences mainly restricted to the loops (Fig. 5A). The high level of overall sequence identity among Stats from multiple species, coupled with the availability of the crystal structure of human Stat1 (24) allowed us to model the SH2 domain of human Stat5b using Swiss Model, a program that predicts structures reliably (root mean square deviation < 2 Å) for sequences with at least 50% identity. The modeled Stat5b shows a structural organization similar to human Stat1 but contains a longer loop between the α2 and α3 helices (Fig. 5B). These two α helices flank a central β-sheet (β1 to β3 in Fig. 5A) that creates a positively charged pocket in the SH2 domain through an invariant arginine (amino acid 618 in human Stat5b and residue 34 in Fig. 5A). The pocket is essential for the dimerization of two Stat5b molecules, which occurs through reciprocal interactions between the SH2 domain on one Stat5b and the phosphotyrosine (amino acid 699 in human Stat5b) on the other (2). Dimerization is stabilized by the ionic interaction between a bidentate ion pair contributed by arginine 618 and two of the oxygen molecules of phosphotyrosine 699. The A630P mutation maps within this conserved β-sheet (Fig. 5B in green), where an alanine is found in Stat5a, 5b, 6, a threonine in Stat1, 3, and 4, and a serine in Stat2 (Fig. 5A). In addition to its key role in protein-protein interactions between two Stat5b molecules, this central β-sheet may be an essential part of the nucleation core for folding, and the presence of a proline at this position may affect both initiation of folding and protein stability. Based on the structural conservation within the SH2 domain, mutations within this segment in other Stats also may make them prone to aberrant folding and aggregation.

Although mis-sense mutations in a large number of human proteins may cause improper folding, only a limited subset have been shown to escape the surveillance mechanisms that would otherwise cause their degradation (35, 36). This group includes mutant versions of several membrane-spanning transporters and receptors that use the secretory pathway, such as the cystic fibrosis transmembrane conductance regulator and rhodopsin, which are prone to aggregation secondary to misfolding (35, 37). A few intracellular proteins with mutations that lead to
addition of long polyglutamine tracts, such as those that cause several chronic neurodegenerative disorders, including Huntington’s disease and spinocerebellar ataxia, also are susceptible to aggregation (38). This latter class includes an aberrant version of the androgen receptor with an expansion of glutamine residues that causes the X-linked inherited disease spinal bulbar muscular atrophy (39). For this disorder, no natural variants of transcription factors have been shown to be prone to protein aggregation. A substitution mutation in the pituitary transcriptional activator Pit-1 has been found to become mislocalized within the nucleus when overexpressed, possibly because of changes in its structure, although no structural determinations have been performed (40).

Larger proteins generally fold in modules, and folding within each segment takes place relatively independently (33). The fully native conformation is only acquired when all native-like interactions are formed within and between domains (33). Many misfolded proteins inappropriately expose hydrophobic surfaces that normally are buried in the interior of the protein (26). Integral membrane proteins such as the cystic fibrosis transmembrane conductance regulator and rhodopsin possess multiple regions of hydrophobic amino acids and thus may be more prone than other nonmembrane proteins to adopt alternative non-native conformations that interact to form aggregates (26). Stat5b(ΔE30P) appears to be the exception to this generalization, because it is a cytoplasmic protein and lacks extensive stretches of hydrophobic amino acids. Although normally the ubiquitin-proteasome system selectively degrades damaged and misfolded proteins, thus protecting cells from potentially toxic effects of protein aggregation, once abnormally folded proteins aggregate, they are not degraded efficiently but rather accumulate and may even impair ubiquitin-proteasome function (30, 31), as we observe with Stat5b(ΔE30P). Aggresomes are considered to be a specific and active cellular response to excessive concentrations of aberrantly folded and aggregated proteins and may be similar to inclusion bodies seen in neurodegenerative diseases (41, 42). Sequestration into aggresomes or inclusion bodies may be part of a protective mechanism, serving to limit but not prevent proteasome dysfunction (30, 43).

The Stat5b(ΔE30P) mutation represents the first example of a disease-causing alteration in a transcription factor in which the fundamental pathogenic mechanism has been shown to be aberrant folding within a specific domain of the protein. This defect not only creates primary Stat5b deficiency with growth failure through impaired GH-stimulated IGF-I expression but also potentially leads to secondary abnormalities as a result of inhibition of proteasome function. Because Stats have been implicated in a wide range of physiological and pathophysiological processes (2, 3), it may be anticipated that analogous mutations in other members of this family will be identified in human diseases.

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