on the absorption cross section, a single molecule requires 10^9 incident photons for one photon to be absorbed, but the presence of a single exciton of the appropriate characteristics is capable of causing this same effect. Second, the combination of single-molecule excitation with an exciton source and a pipette that can be scanned with high resolution across a surface leads to a new way of imaging with light that surpasses any presently available methodologies. Specifically, if one scans such an exciton source across a surface, exciton transfer to the surface will occur only when a point in the crystal is within about 8 nm of an acceptor in the surface. Thus, by detecting the acceptor's emission or a reduction in the intensity of the donor's emission as a function of the position of the exciton source, an image with molecular resolution should be obtainable with light. This form of imaging, which we call molecular exciton microscopy (MEM), should have wide applicability to nondestructive imaging of biological membranes and other molecular surfaces at ambient conditions.

Such sources should also be important as an analytical chemical tool for microanalysis and sensing with light that bypasses absorption path-length requirements. A specific example of such an application for these exciton sources is capillary zone electrophoresis (*19*). In this technique molecules are separated by electric fields along a glass capillary. In principle, the smaller the dimension of the capillary, the better the separation. However, part of the limitations encountered by this technique are absorption path-length requirements for the detection of the separated species. Exciton sources and the methodology of exciton excitation outlined here conceivably could alleviate some of these problems.

Finally, it is instructive to compare the dimensionalities, the ease of fabrication, and the approach to light excitation described here to those presently in use in integrated optics. The smallest light sources available today for integrated optics are light-emitting diodes and diode lasers, which have the potential to be used as light sources, switches, modulators, and detectors. These devices are fabricated by means of sophisticated molecular beam epitaxy techniques that involve multimillion-dollar instrumentation. Even with such instrumentation, the typical devices constructed have wavelengths beyond 670 nm and have rectangular rather than cylindrical symmetry, with initial spot sizes of 2 by 5 μm (*20*). This rectangular structure causes significant astigmatism with parallel and perpendicular parts of the beam acting as if they come from two different sources separated by 40 μm (*20*). These beam characteristics of diodes waste much light when coupled to the circular entrance of an optical fiber. In contrast, our exciton sources have circular beams and the fact that the crystals used in such sources have been made to lase is also important for possible applications in integrated optics. Because of the high efficiency of exciton generation, electrical excitation of such crystals may make it possible to achieve laser action in the confined space of a pipette (which has a structure that could be ideal for laser action). The methodologies described in this report have much potential for use in molecular exciton microscopy, molecular sensing, or as a cheap and compact short wavelength light source.

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Evidence for a Novel Thioredoxin-Like Catalytic Property of Gonadotropic Hormones

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It has been proposed that dithiol-disulfide interchange and oxidation-reduction reactions may play a role in hormone-induced receptor activation. Inspection of the sequences of the gonadotropic hormones revealed a homologous tetrapeptide (Cys-Gly-Pro-Cys) between the β subunit of lutropin (LH) and the active site of thioredoxin (TD). The β subunit of follitropin (FSH) has a similar sequence (Cys-Gly-Lys-Cys). Thioredoxin is a ubiquitous protein serving as an electron donor for ribonucleotide reductase, but it also exhibits disulfide isomerase activity. The catalytic activity of TD was assayed by its ability to reactivate reduced and denatured ribonuclease. In this assay, the purified ovine FSH and bovine LH preparations tested were ~60 and ~300 times, respectively, as active as TD on a molar basis. This heretofore unsuspected catalytic property of FSH and LH may be important in understanding their mechanism of receptor activation and signal transduction.

FOLLITROPIN (FSH) AND LUTROPIN (LH) are complex, heterodimeric glycoprotein hormones [relative molecular mass (M_r) of ~35,000] secreted by the anterior pituitary gland and are responsible for normal gonadal development and function (*1–3*). These disulfide-rich hormones share a common α subunit, with biological specificity conferred by their β subunits (*1–3*). Recently, the LH receptor from porcine testis (*4*) and rat ovaries (*5*) has been cloned, and the FSH receptor has been purified from bovine calf testis (*6*). Signal transduction for FSH and LH occurs via the adenosine 3′,5′-monophosphate (cAMP) second-messenger system (*1–3*), and the nature of the hormone-receptor interaction has been the subject of intense study (*3*). Nevertheless, the molecular events leading to the activation of gonadotropic hormone receptors after interaction with FSH or LH remain obscure.

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Robillard and Konings (*7*) suggested that dithiol-disulfide interchange could play a role in receptor activation. Oxidation-reduction (redox) reactions between ligands and receptors have been proposed to occur in insulin (*8–10*), prolactin (*11*), prostaglandin (*12*), and catecholamine (*13, 14*) systems. Inspection of the amino acid sequences of the glycoprotein hormones revealed a homologous tetrapeptide between the β subunit of LH and the active site of thioredoxin (TD) (Fig. 1). A similar sequence exists in the β subunit of FSH (Fig. 1). Protein disulfide isomerase (PDI) also has a similar sequence (Cys-Gly-His-Cys) associated with its active site (*15*). These vicinyl Cys residues of TD and PDI undergo reversible oxidation to form disulfide bonds and are responsible for the redox activity of these proteins (*16, 17*). Thioredoxin is a ubiquitous protein (M_r, ~12,000), generally considered to function as an electron donor for ribonucleotide reductase (*17*). Also, TD has shown PDI activity, as determined by its ability to catalyze the reactivation of reduced and denatured ribonuclease (rRNase) and of ribonuclease (RNase) that is scrambled in that it contains incorrectly paired disulfide bonds (*18*). Intrinsic TD or PDI activity of LH and FSH, if present, would suggest a novel mechanism for ligand modification of receptor conformation, which could be of importance in understanding receptor activation and signal transduction.

A search of the Protein Identification Resource (PIR) database indicated that 205 of 5556 sequences (3.7%) contained a Cys-X-X-Cys motif. Many of these are the same protein from different species and the same protein from the same species reported by different laboratories. When the search was restricted to the Cys-Gly-Pro-Cys sequence, only nine proteins of 5556 sequences were found to contain this homology, of which LH was the only hormone. A conserved substitution (Arg for Lys) exists adjacent to the Cys-X-X-Cys motif in LH-β (Fig. 1). It is thought that TD reduces protein disulfide bonds through the formation of a thiol anion stabilized by the positive charge of this vicinal Lys residue (*17*). An adjacent Arg residue, as found in the LH-β subunit, should similarly stabilize a thiol anion. When the PIR database was searched for Cys-X-X-Cys-(Arg or Lys), 13 proteins were positive, of which LH and corticoliberin are the only hormones. Additionally, at least one active form of TD (from cyanobacterium) exists with an Arg for a Lys substitution at this site (*19*). The homologous tetrapeptide sequence in LH-β, together with the neighboring Arg, exists without substitution in LH of several species.

To investigate the similarities between the active site of TD and the homologous sites in LH and FSH, we compared their hydropathy profiles and predicted secondary structure (Fig. 2) (*20*). X-ray crystallography of TD indicates that the active site is located in a solvent-exposed β-turn (*21*). Secondary structure assessment correctly predicted this β-turn in TD. Despite the known accessibility of the active site, cysteines in TD are located in a neutral hydropathic region (Fig. 2). Analysis of the β subunits of LH and FSH indicated that the sequences of interest are hydrophilic and predicted to be located in a β-turn, despite the amino acid substitutions at, and adjacent to, the TD-like sites. These observations (Fig. 2) suggest that the TD-like sequences in FSH-β and LH-β are exposed to solvent, accessible for reactions with protein substrates, and similarly associated with β-turn structure. Although disulfide assignments among the gonadotropic hormones remain controversial, the placement of a disulfide at this site has been proposed for the β subunit of FSH (*22*).

The reactivation of rRNase in the presence and absence of TD, LH, or control proteins is shown in Fig. 3. Recovery of activity was about 20% after 100 hours in the absence of catalyst. However, in the presence of 500 μ*M* TD, quantitative recovery of RNase activity is obtained after ~30 hours. The $t_{1/2}$ (time in hours required to recover 50% of RNase activity) at this concentration of TD is ~12 hours, a value comparable with that previously reported (8 hours) (*18*). The reactivation of rRNase is also catalyzed by 1 μ*M* LH with quantitative recovery of RNase occurring by ~30 hours (Fig. 3). The bovine LH preparation tested (*22a*) was, therefore, ~300 times more active than TD on a molar basis in this assay. Reactivation in the presence of control proteins [bovine serum albumin (BSA), ovalbumin, γ-globulin, insulin, or prolactin (1 μ*M* each)] was not greater than 25% after 48 hours. Proteins such as BSA, ovalbumin, and γ-globulin, which contain more disulfide bonds than LH, were less efficient than LH at catalyzing RNase reactivation. Thus the greater number of disulfide bonds in LH than TD (11 versus 1) cannot account for the greater activity of LH in this assay. Additionally, when LH was heated to 70°C for 15 min, its TD-like activity was markedly (>80%) reduced, indicating that the activity was dependent on the structural integrity of the hormone.

The reactivation of rRNase as a function of various concentrations of LH and FSH (Fig. 4) shows a direct relation between the concentration of hormone and the rate of rRNase refolding. RNase activity was recovered quantitatively at concentrations of LH of 0.3 μ*M* or greater (Fig. 4A). Significant stimulation of RNase recovery occurred in the presence of as little as 0.02 μ*M* LH. FSH also catalyzes the reactivation of rRNase, and complete recovery of activity

Fig. 1. Sequence homologies. Alignment of the redox-active Cys residues of TD [identical in many species, for partial list see (*33*)] and rat PDI (*15*) with the ninth and tenth Cys of the β subunit of LH and FSH. The amino acid sequences shown for the hormones are conserved for several species (ovine bovine, porcine), whereas human LH contains an Arg for His substitution. The residue numbers corresponding to these amino acids in the hormones differ slightly depending on species, and, for simplicity, locations are defined according to which Cys (of a total of 12) they represent. For sequence numbers see (*1*).

TD	–Trp–Cys–Gly–Pro–Cys–Lys–
PDI	–Trp–Cys–Gly–His–Cys–Lys–
LH-β	–His–Cys–Gly–Pro–Cys–Arg–
FSH-β	–His–Cys–Gly–Lys–Cys–Asp–

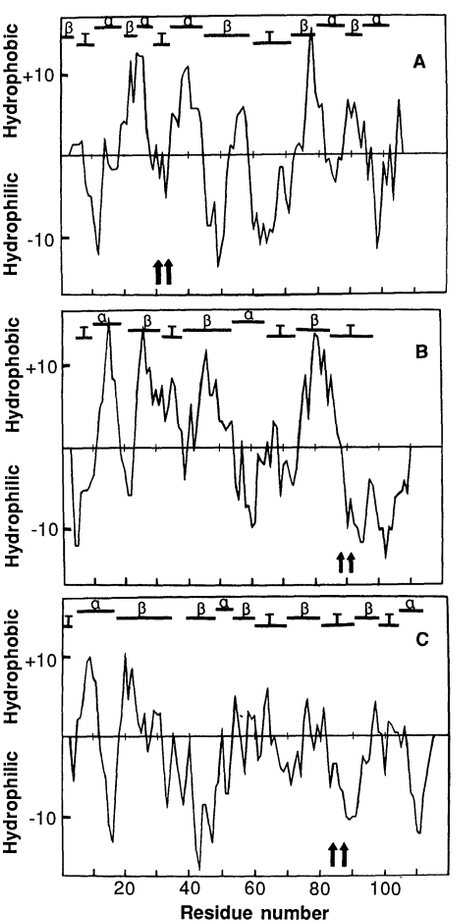

Fig. 2. Hydropathic and secondary structure assessment. Hydropathy profiles (*20*) of *Escherichia coli* TD (**A**) and the β subunits of human LH (**B**) and FSH (**C**). Plotted on the ordinate is the hydropathic value. Bars superimposed on the top of each panel are the results of secondary structure predictions (*20*). Structure predictions are designated as follows: α, α helix; β, β sheet; and T, turn. The arrows denote the positions of the Cys residues of interest.

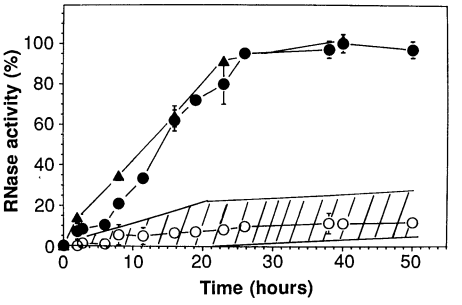

Fig. 3. Reactivation of reduced and denatured RNase. Illustrated is the reactivation in the absence of a catalyst (○), in the presence of 1 μ*M* BSA, ovalbumin, γ-globulin, insulin, or prolactin (hatched region), or in the presence of 1 μ*M* LH (●) or 500 μ*M* TD (▲). Reduced and denatured RNase was prepared fresh for each experiment as described by Pigiet and Schuster (*18*) and was reactivated as described in (*18*), by first diluting rRNase to a final concentration of 400 μg/ml in 0.1*M* tris-HCl (*p*H 7.4) with 1 m*M* EDTA containing either hormone (*22a*), TD, or protein controls. Reactivation was performed in 1.5 ml of polypropylene microfuge tubes at 25°C, and the extent of reactivation was monitored by removing aliquots at various time intervals and measuring RNase activity. Activity was expressed as a percentage of the activity of a native RNase control. RNase activity was determined by the method of Crook *et al.* (*34*), except that the final assay mixture consisted of 1.4 μ*M* RNase and 0.44 m*M* cytidine 2′,3′-monophosphate (cCMP) in 0.1*M* 2-(*N*-morpholino)propanesulfonic acid (MOPS) (*p*H 7.0). Changes in absorbance at 284 nm were monitored with a Gilford Model 250 Spectrophotometer. The results shown are the means ± SD of triplicate experiments.

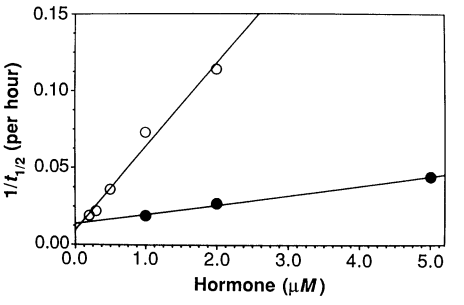

Fig. 5. Comparative potency and dose dependency of LH- and FSH-catalyzed RNase reactivation. Plotted is the hormone concentration as a function of the reciprocal of the time required to regain 50% RNase activity ($t_{1/2}$). These data were taken from Fig. 4. LH, ○; FSH, ●.

occurs by 80 hours at FSH concentrations of 1 μ*M* and greater (Fig. 4). Hormone-catalyzed reactivation of rRNase is characterized by an early lag phase, which becomes less pronounced at higher concentrations, as is true with TD (*18*). A plot of the concentrations of hormone as a function of the reciprocal of the $t_{1/2}$ indicates a linear dose dependency (Fig. 5). Since 1 μ*M* FSH is equivalent in catalytic activity (same $t_{1/2}$) to 0.20 μ*M* LH, the ovine FSH preparation tested (*22a*) is taken to be approximately 20% as active as LH, or ~60 times more active than TD on a molar basis. Several different preparations of bovine LH and ovine FSH, as well as purified preparations of porcine and human LH and FSH, were also tested in the rRNase assay. Each had activity significantly greater than TD on a molar basis.

Involvement of several of the 11 disulfides of FSH during the catalysis of rRNase refolding would likely result in structural changes in the hormone and an altered receptor-binding potency. However, receptor-binding potency of FSH was unchanged after catalyzing the complete refolding of rRNase, indicating that the hormone retained, postcatalytically, the native conformation essential for receptor binding. Thus the greater activity of the hormones than TD in this assay is probably not the result of involvement of many active disulfides, but rather of only one or a few active sites.

The TD-like activity in the gonadotropic hormones may be involved in generating the active hormone-receptor complex. Such intrinsic TD-like activity could also play a role in catalyzing refolding of newly synthesized subunit molecules, or in the process of gonadotropin subunit association. The TD-like sequences of the hormones are immediately adjacent to the "determinant loop" region proposed by Gordon and Ward (*23*) as being responsible for conferring biological specificity to these hormones. The receptors for the glycoprotein hormones appear to be composed of subunits that are joined by disulfide bonds (*3, 24–27*) and may contain free sulfhydryl groups in a region of hormone binding (*28*). Also, a membrane equilibrium has been postulated to exist between LH receptor monomer and disulfide-linked oligomer (*27*). Thus the receptor, as the natural substrate for the hormone, contains groups that are potentially active in such redox reactions. Glycoprotein hormone binding to receptors becomes increasingly irreversible as a function of time or temperature (*29–31*). One possible explanation for this irreversibility is hormone-receptor disulfide interchange. A TD-like active site in the hormones could be responsible for disulfide cross-linking leading to formation of an irreversible hormone-receptor complex. Recently, an autocrine factor associated with interleukin-2 receptor up-regulation after human T cell lymphotropic virus (HTLV-I) transformation of T cells was found to be similar, if not identical, to human TD, and its effect apparently required the reduced form of TD protein (*32*). Such observations, together with the results reported here for LH and FSH, suggest a novel mechanism of ligand-induced receptor activation, whereby disulfide-isomerization or redox reactions produce structural changes in the receptor required for signal transduction.

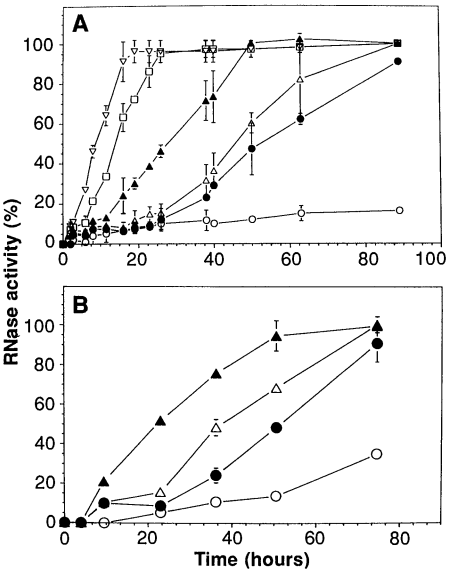

Fig. 4. Kinetics of reactivation of rRNase as a function of the concentration of LH and FSH. (**A**) The percent reactivation in the absence of a catalyst (○) or the presence of 0.2 μ*M* (●), 0.3 μ*M* (△), 0.5 μ*M* (▲), 1.0 μ*M* (□), or 2.0 μ*M* (▽) LH (*22a*). (**B**) Similar data obtained in the absence of a catalyst or the presence of 1.0 μ*M* (●), 2.0 μ*M* (△), or 5.0 μ*M* (▲) FSH (*22a*). Experimental protocols were as described in the legend to Fig. 3. The data shown are the means ± SD of duplicate experiments.

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High-Resolution Mapping of Human Chromosome 11 by in Situ Hybridization with Cosmid Clones

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Cosmid clones containing human DNA inserts have been mapped on chromosome 11 by fluorescence in situ hybridization under conditions that suppress signal from repetitive DNA sequences. Thirteen known genes, one chromosome 11–specific DNA repeat, and 36 random clones were analyzed. High-resolution mapping was facilitated by using digital imaging microscopy and by analyzing extended (prometaphase) chromosomes. The map coordinates established by in situ hybridization showed a one to one correspondence with those determined by Southern (DNA) blot analysis of hybrid cell lines containing fragments of chromosome 11. Furthermore, by hybridizing three or more cosmids simultaneously, gene order on the chromosome could be established unequivocally. These results demonstrate the feasibility of rapidly producing high-resolution maps of human chromosomes by in situ hybridization.

EFFORTS TO CONSTRUCT A HIGH-RESolution map of the human genome have intensified significantly during the past few years. Analysis of the inheritance of restriction fragment length polymorphisms in family pedigrees has permitted the construction of linkage maps for most regions of the human genome (*1*) as well as the identification of the genetic loci for many diseases (*2*). Physical mapping techniques provide a complement to genetic linkage. Standard agarose gel electrophoresis gives resolution in the 1- to 10-kb range, and pulsed field techniques extend this range to the megabase level. However, gel electrophoresis methods are not useful for producing an initial localization for a DNA sequence that has not been mapped previously. Furthermore, they do not directly provide the ability to order DNA sequences more than a few hundred kilobases apart or the ability to assign DNA sequences to specific chromosomal regions. For initial chromosome assignment and precise gene localization, somatic cell genetics, fluorescence-activated cell sorting of metaphase chromosomes, and in situ hybridization have been widely used.

The most direct method for identifying the chromosomal locus of a segment of human DNA is by in situ hybridization. Although unique sequences less than 1 kb long can be localized by using isotopically labeled probes, autoradiographic development times are long (often weeks or months), extensive statistical analysis is required, and the mapping precision is limited by the necessity of having to capture the emitted isotopic signal by an emulsion overlay. In contrast, nonisotopically labeled probes offer markedly improved speed and spatial resolution, but in general they have suffered from a lack of sensitivity. However, several groups have reported the detection of unique sequence targets of 6 kb or less by nonisotopic in situ hybridization (*3–5*).

Previous studies have demonstrated that it is possible to suppress the hybridization signal from ubiquitous repeated sequences, such as the Alu and Kpn elements, by adding appropriate competitor DNAs to the probe mix. Such suppression strategies, which exploit the rapid reassociation kinetics of repetitive sequences, have been used to facilitate the selective hybridization of unique sequence subsets from probes for Southern blotting (*6*) and for in situ hybridization (*5, 7–10*). In this report, we describe the use of chromosomal in situ suppression (CISS) hybridization (*11*) in conjunction with fluorescent detection of hybridized probes for the rapid and precise localization of large numbers of cloned genomic DNA segments and for the development of a physically based map of human chromosome 11. Furthermore, a detailed comparison between results obtained by CISS hybridization and map order derived by somatic cell genetics reveals a one to one correspondence between the two methods.

To determine the chromosome to which a probe has hybridized, a technique for chromosome identification compatible with CISS hybridization is required. Conventional Giemsa banding of chromosomes before hybridization lowers hybridization efficiency, whereas posthybridization staining quenches fluorescence. Although it is possible to do Giemsa banding after hybridization (*4*), this process requires the relocalization of specific metaphase spreads and two separate photographic steps. However, alternative banding or labeling methods for direct chromosome identification are available. For example, quinacrine banding, bromodeoxyuridine banding, and diamidinophenylindole banding are compatible with fluorescent detection of probes by in situ hybridization (*12*). As an alternative strategy we have used cohybridization with a differently labeled probe (or probe set), such as an Alu DNA BLUR clone (*13*), which gives a banding pattern resembling R banding (*14*), or with probes tagging a particular chromosome such as a previously mapped cosmid clone or a chromosome-specific DNA repeat (*12, 15*). A DNA library from a sorted human chromosome that decorates the cor-

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